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THE TOXICOLOGY, ENVIRONMENTAL FATE, AND HUMAN RISK OF HERBICIDE ORANGE AND ITS ASSOCIATED DIOXIN

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The use of herbicides in South Vietnam between 1962 and 1971 was reviewed, including the nature and quantities of herbicides used, their handling and application. Emphasis was placed on Herbicide Orange, a 50:50 mixture of 2,4-dichlorophenoxyacetic acid (2,4-D) and 2,4,5-trichlorophenoxyacetic acid (2,4,5-T), with its associated contaminant 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD). The at-risk US military population in South Vietnam was defined to establish the potential for exposure in handling and application of Herbicide Orange. The environmental fate of the phenoxy herbicides and TCDD was reviewed to evaluate

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the potential for human risk associated with exposure to areas previously treated with Herbicide Orange. The occupational and environmental aspects of the project to incinerate at sea 2.22 million gallons of Herbicide Orange during the summer of 1977 were summarized to assess the potential for human exposure in handling large quantities of the material. Scientific data were reviewed on incidents and episodes involving suspected poisoning of humans or animals by phenoxy herbicides or TCDD. Literature dealing with animal toxicology and the effects of human exposure to 2,4-D, 2,4,5-T and TCDD was reviewed to correlate exposures with symtomatology.

PREFACE

The use of herbicides in support of tactical military operations in South Vietnam from 1961 to 1971 has had (and continues to have) a negative impact on the use of pesticides by numerous facets of our society. Prior to the Vietnam conflict, herbicides were considered invaluable to agriculture, innocuous to human life and of little environmental concern. Today, seven years after the last herbicide mission in Vietnam, these same herbicides are the center of scientific debate involving not only ecological but also medical, legal and political issues. The United States Environmental Protection Agency (EPA) has recently issued a Notice of Rebuttable Presumption Against Registration (RPAR) of pesticides containing one of these "Vietnam" herbicides, while at the same time some Veterans of the Vietnam Conflict have reported medical problems they claimed were the result of herbicide exposure while assigned to military duties in Vietnam.

In April 1978, the Surgeon General of the United States Air Force (USAF) tasked personnel of the USAF Occupational and Environmental Health Laboratory, Brooks AFB, Texas with updating previous scientific assessments of possible adverse effects to human health resulting from exposure to the herbicides, especially Herbicide Orange used in South Vietnam during the Vietnam Conflict.

The present report was assembled using the latest available published scientific information, previously unpublished data, and observations from medical and scientific personnel intimately associated with the herbicides in question. The report reviews the use of phenoxy herbicides in Vietnam, their environmental fate, and pertinent animal and human toxicological studies. In addition, a description is given of the 1977 military operation for the disposal of Herbicide Orange emphasizing the facets of environmental monitoring and industrial hygiene. The document concludes with an assessment of the risk to human health following exposures to the phenoxy herbicides. Special emphasis was placed upon the chemistry, environmental fate and toxicology of the trichlorophenoxy herbicide contaminant, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD or dioxin).

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CHAPTER I
THE USE OF HERBICIDES IN SOUTH VIETNAM

I. INTRODUCTION

The introduction of herbicides in 1962 into the armed conflict in Vietnam represented an application of a new technique for modern warfare. Their use in a defensive role was for defoliation. Their use in offensive roles was for food crop denial. The herbicides most widely employed were the phenoxyacetic acids. They were extensively used for almost a decade throughout the forested, semi-populated, regions of South Vietnam. Assessments of their ecological impact in South Vietnam have been published (see Chapter V). An assessment of the effects of herbicides on the human indigenous populations of South Vietnam has also been conducted (11). No assessment has been made of potential adverse human effects of the phenoxy herbicides or the toxic contaminant 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) on personnel of the U.S. Military forces.

Adverse human effects in military personnel due to the herbicides or the contaminant would be predicated on the assumption that an exposure occurred. The presence of military spray aircraft or the observation that drums of herbicide were stored on a military installation, or even smelling the odor of "herbicides" in the air does not necessarily constitute an exposure to the herbicide per se. An exposure would have had to involve physical contact for a sufficient period of time to permit the chemical(s) to penetrate the body. This chapter examines those factors that would have influenced the likelihood of such exposures. They include:

1. the nature of the herbicides used in South Vietnam,

2. the nature of the herbicide applications,

3. the procedures employed in the handling of the herbicides, and

4. the quantities of individual chemicals sprayed in South Vietnam.

Detailed examinations of these "parameters" are reported in the following sections.

II. THE HERBICIDES USED IN SOUTH VIETNAM

A. Historical

The discovery and early history of the phenoxy herbicides 2,4-dichlorophenoxyacetic acid (2,4-D) and 2,4,5-trichlorophenoxyacetic acid (2,4,5-T) have been reviewed by Peterson (31). Peterson noted that the effectiveness of these plant growth regulators as "herbicides" was determined in mid-1944 field trails at Beltsville and Camp Detrick (now Fort Detrick), Maryland. The outstanding effectiveness of these two herbicides in controlling the growth of broad-leaved plants and weeds, coupled with their apparently low mammalian toxicity and low application rates, resulted in their rapid acceptance in world agriculture. Peterson (31) reported that the annual production of 2,4-D alone exceeded 14,000 pounds in 1950 and 36,000,000 pounds in 1960.

Irish et al (26) and Darrow et al (14) have documented the early military use of the phenoxy herbicides. They reported that the earliest aerial spray trials (conducted in military aircraft) occurred in 1944 and 1945. Three different mixtures of 2,4-D were used in these early tests. Although herbicides were not used in tactical military operations in World War II, a small program for screening potential herbicides for military use continued after the War. By 1951, personnel at Fort Detrick had determined that the vegetation-control chemicals of choice were mixtures of the butyl esters of 2,4-D and 2,4,5-T. In 1959, the Crops Division, Fort Detrick, conducted the first large-scale military defoliation effort at Fort Drum, New York. This project involved the aerial application of the butyl esters of 2,4-D and 2,4,5-T to approximately four square miles of vegetation. Its success prompted the Office of the Secretary of Defense (OSD) in May 1961 to request that the Crops Division determine technical feasibility of defoliating jungle vegetation in the Republic of Vietnam. As part of a project to evaluate herbicides and defoliation techniques (Project AGILE) in Southeast Asia, Brown (7) conducted eighteen different aerial spray tests (defoliation and anticrop) with various formulations of commercially available herbicides. The choice of these herbicides was based "upon the chemicals that had had considerable research, proven performance, and practical background. Also, other factors had to be considered, such as availability in large quantity, costs and known or proven safety in regard to their toxicity to humans and animals" (7). The results of these tests were that significant defoliation and anticrop effects could be obtained with two different mixtures of herbicides. The first was a mixture of the n-butyl esters of 2,4-D and 2,4,5-T and the iso-butyl ester of 2,4,5-T. This mixture was code-named "Purple". The second "military" herbicide was code-named "Blue" and consisted of the acid and sodium salt of cacodylic acid. The colored bands which were painted around the center of the 55-gallon drums served as aid to the identification by support personnel.

Brown (7) reported that the first shipment of Herbicides Purple and Blue was received at Tan Son Nhut Air Base, Republic of Vietnam, on 9 January 1962. These were the first military herbicides used in Operation RANCH HAND, the tactical military project for the aerial spraying of herbicides in South Vietnam. Two additional phenoxy herbicide formulations were received in limited quantities in South Vietnam and evaluated during the first two years of Operation

RANCH HAND. These were code-named Pink and Green and will be described in the subsequent section. By January 1965, two additional military herbicides had been evaluated and brought into the spray program. These were code-named Orange and White, and are also described below. Herbicide Orange replaced all uses of Purple, Pink, or Green and eventually became the most widely used military herbicide in South Vietnam.

In April 1970, the Secretaries of Agriculture; Health, Education and Welfare, and the Interior jointly announced the suspension of certain uses of 2,4,5-T. These suspensions resulted from published studies indicating that 2,4,5-T was a teratogen. Subsequent studies revealed that the teratogenic effects had resulted from a toxic contaminant in the 2,4,5-T, identified as 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD). Subsequently, the Department of Defense suspended the use of Herbicide Orange (4). At the time of the suspension, the Air Force had an inventory of 1.37 million gallons of Herbicide Orange in South Vietnam and 0.85 million gallons at the Naval Construction Battalion Center (NCBC), Gulfport, Mississippi. In September 1971, the Department of Defense directed that the Herbicide Orange in South Vietnam be returned to the United States and that the entire 2.22 million gallons be disposed of in an environmentally safe and efficient manner. The 1.37 million gallons were moved from South Vietnam to Johnston Island, Pacific Ocean for storage in April 1972.

B. Descriptions of the Herbicides Used in Operation RANCH HAND

The following military herbicides were used in South Vietnam in Operation RANCH HAND. The first three were extensively used in both defoliation and anticrop programs. Only limited quantities of the herbicides Purple, Pink or Green were used in South Vietnam, and then primarily during the 1962-1964 time period.

1. Herbicide Orange

Orange was a reddish-brown to tan colored liquid soluble in diesel fuel and organic solvents, but insoluble in water. One gallon (gal) of Orange theoretically contained 4.21 pounds (lb) of the active ingredient of 2,4-D and 4.41 lb of the active ingredient of 2,4,5-T. Orange was formulated to contain a 50:50 mixture of the n-butyl esters of 2,4-D and 2,4,5-T. The percentages of the formulation typically were:

n-butyl ester of 2,4-D	49.49
free acid of 2,4-D	0.13
n-butyl ester of 2,4,5-T	48.75
free acid of 2,4,5-T	1.00
inert ingredients (e.g., butyl alcohol and ester moieties)	0.62

Some of the physical, chemical, and toxicological properties of Orange are listed in Table 1. The structures of the n-butyl esters of 2,4-D and 2,4,5-T are shown in Figure 1.

2. Herbicide White

White was a dark brown viscous liquid that was soluble in water but insoluble in organic solvents and diesel fuel. One gal of White contained 0.54 lb of the active ingredient of 4-amino-3,5,6-trichloropicolinic acid (picloram) and 2.00 lb of the active ingredient of 2,4-D. White was formulated to contain a 1:4 mixture of the triisopropanolamine salts of picloram and 2,4-D. The percentages of the formulation were:

triisopropanolamine salt of picloram	10.2
triisopropanolamine salt of 2,4-D	39.6
inert ingredient (primarily the solvent triisopropanolamine)	50.2

Some of the physical, chemical, and toxicological properties of White are listed in Table 1. The structures of the triisopropanolamine salts of 2,4-D and picloram are shown in Figure 1.

3. Herbicide Blue

Blue was a clear yellowish-tan liquid that was soluble in water, but insoluble in organic solvents and diesel fuel. One gal of Blue contained 3.10 lb of the active ingredient hydroxydimethyarsine oxide (cacodylic acid). Blue was formulated to contain both cacodylic acid (as the free acid) and the sodium salt of cacodylic acid (sodium cacodylate). The percentages of the formulation were:

cacodylic acid	4.7
sodium cacodylate	26.4
surfactant	3.4
sodium chloride	5.5
water	59.5
antifoam agent	0.5

Some of the physical, chemical, and toxicological properties of Blue are listed in Table 1. The structure of the sodium salt of cacodylic acid is shown in Figure 1. It should be noted that cacodylic acid and sodium cacodylate contained arsenic in the form of the pentavalent, organic arsenical. This form of arsenic was essentially nontoxic to animals as can be noted by the LD_{50} value for white rats. Of the total formulation, 15.4 percent was arsenic in the organic form, only trace quantities were present in the inorganic form. The term Herbicide Blue was first applied to powdered cacodylic acid in 1961 through 1964. This first Herbicide Blue contained 65 percent active ingredient

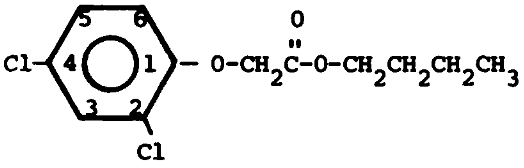
TABLE 1. Selected physical, chemical and toxicological properties of the three major military herbicides used in South Vietnam, 1962 - 1971.[a].

Herbicide Code Name	Molecular Mass	Specific Density, 25°C	Viscosity, Centipose, 23°C	Weight Total Ester lb/gal	Weight Acid Equivalent lb/gal	Soluble in Water	Specific Toxicity for White Rats mg/kg[b].	Relative Toxicity
Orange	589	1.28	43	10.7	8.62	No	566	Low
White	1,173	1.12	125	9.4	2.54	Yes	3,080	Very Low
Blue	296	1.32	14	10.9	3.10	Yes	2,600	Very Low

[a] Source: (35)

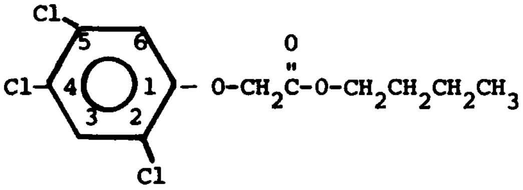
[b] Milligrams of the herbicide per kilogram of body weight of the test animal lethal to 50 percent of white rats.

A.


Cl
Cl
O-CH2C-O-CH2CH2CH2CH3
O

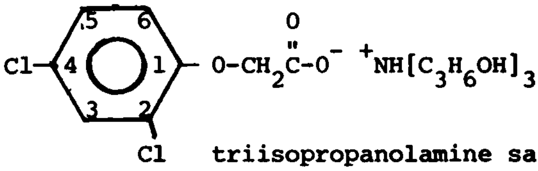
n-butyl ester of 2,4-dichlorophenoxyacetic acid (2,4-D)

B.


Cl
Cl
Cl
O-CH2C-O-CH2CH2CH2CH3
O

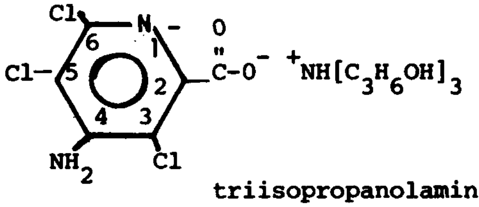
n-butyl ester of 2,4,5-trichlorophenoxyacetic acid (2,4,5-T)

C.


Cl
Cl
O-CH2C-O⁻ ⁺NH[C3H6OH]3
O

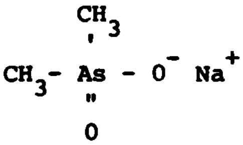
triisopropanolamine salt of 2,4-D

D.


Cl
Cl
Cl
N
NH2
C-O⁻ ⁺NH[C3H6OH]3
O

triisopropanolamine salt of 4-amino-3,5,6-trichloropicolinic acid (picloram)

E.



$$CH_3-\underset{O}{\overset{CH_3}{As}}-O^-\ Na^+$$

sodium salt of hydroxydimethylarsine oxide (cacodylic acid)

FIGURE 1. Chemical structure and nomenclature of the major herbicides used in South Vietnam, 1962-1971. Formulas A and B comprised Orange, C and D - White, and E was Blue.

cacodylic acid and 30 percent sodium chloride and was mixed in the field with water (7, 14).

4. Herbicide Orange II

Orange II was the code-name of a formulation similar to Orange with the difference being the substitution of the issocytl ester of 2,4,5-T for the n-butyl ester of 2,4,5-T. The physical, chemical, and toxicological properties of Orange II were similar to those of Orange. Orange II was produced solely by one chemical company. Approximately 950,000 gal of Orange II were shipped to South Vietnam during 1968 and early 1969 (12). How much Orange II was returned to Johnston Island from South Vietnam in April 1972 was not determined.

5. Herbicide Purple

Purple was first formulated in the mid-1950s time period. It was used in the Camp Drum, New York, defoliation test in 1959 (26). The formulation was a brown liquid soluble in diesel fuel and organic solvents but insoluble in water. One gal of Purple contained 8.6 lb of the active ingredients 2,4-D and 2,4,5-T. The percentages of the formulation were:

n-butyl 2,4-D	50
n-butyl 2,4,5-T	30
iso-butyl 2,4,5-T	20

The physical, chemical, and toxicological properties of Purple were similar to those described for Orange.

6. Herbicide Pink

Pink was a formulation of 2,4,5-T used extensively in early RANCH HAND operations (7) and in the defoliation test program of 1963 and 1964 in Thailand (15). Pink was a mixture of the n-butyl and iso-butyl esters of 2,4,5-T. No data were available on the physical, chemical, or toxicological properties of Pink. However, Darrow et al (15) reported that it contained 8.16 lb active ingredient per gal. The percentages of Pink formulation were:

n-butyl 2,4,5-T	60
iso-butyl 2,4,5-T	40

7. Herbicide Green

Green was a single component formulation consisting of the n-butyl ester of 2,4,5-T. It was used in limited quantities in the 1962-1964 period (3). However, the only reported use of Green was in an evaluation program of herbicides for use against manioc and [(7), and correspondence between personnel of the Air Force Armament Laboratory, Eglin AFB, Florida, and personnel of the Crops Division, Fort Detrick, Maryland, dated 5 Sep 63]. No data were available on physical, chemical, or toxicological properties of Green. Brown (7) reported that Green contained the same amount of active ingredient as Pink.

8. Other Herbicides Used in South Vietnam

In addition to evaluating Herbicides Purple, Pink and Green, Brown (7) also evaluated Dinoxol, a mixture of 20 percent each of the butoxy ethanol esters of 2,4-D and 2,4,5-T; Trinoxol, 40 percent butoxy ethanol ester of 2,4,5-T; Diquat, 6,7-dihydrodipyridol (1,2-a:2', 1'-C) pyrazidinium dibromide; and small quantities (grams) of 16 different chemicals. The latter chemicals were applied on native grasses and bamboo at the Saigon Navy Yard. Darrow et al (14) reported that small quantities of soil-applied herbicides were used on base camp perimeters, mine fields, ammunition storage areas, and other specialized sites requiring control of grasses and woody vegetation. The soil-applied herbicides evaluated for use in South Vietnam included Bromacil, 5-bromo-3-sec-butyl-methyluracil; Tandex, (3,3-dimethyluneido) phenyl-tert-butylcarbamate; Monuron, 3-(p-chlorophenyl)-1, 1-dimethylurea; Diuron, 3-(3,4-dichlorphenyl)-1, 1-dimethylurea; and Dalapon, 2,2-dichloropropionic acid.

C. Quantities of Herbicides Sprayed in South Vietnam

The estimated number of gal of the various military herbicides sprayed in South Vietnam from 1962 through 1971 have been obtained by examination of procurement and disposition records. The data obtained from these sources were compared to other sources when available; e.g., actual tactical mission records. Table 2 presents a summary of the available data on the number of gal of herbicides Blue, Green, Pink and Purple procured and disseminated in South Vietnam between January 1962 and December 1964. (3) Table 3 gives a comparison of the estimated number of gal procured and disseminated in South Vietnam between January 1965 and February 1971 as reported by the National Academy of Science (11), Westing (34), and Craig (12). The discrepancies in total herbicide quantity between the three sources occurred because Craig's data were from procurement records only, while the NAS and Westing data are based on both records and estimates. The latter two reports used different assumptions in calculating the total herbicide volume. These included such factors as spray line data (length and width of the spray swath), rate of application (1.5 or 3 gal/acre, and the amount of herbicide disseminated during a mission.

TABLE 2. Number of gallons of military herbicide procured by the U.S. Department of Defense and disseminated in South Vietnam during the period January 1962 - December 1964.[a]

Military Herbicide	Gallons of Formulation	Pounds Active Ingredient
Blue[b]	5,200	10,000
Green[c]	8,208	66,980
Pink[c]	122,792	1,001,980
Purple[d]	145,000	1,180,300
Total	281,200	2,259,260

[a] Source document: Memorandum for Assistant Secretary of Defense from the Office of the Under Secretary, Department of the Air Force, Washington, D.C.; dated December 15, 1961. Subject: Summary of Current Status Project "RANCH HAND" Chemicals. (3)

[b] Blue was procured as a fine white hygroscopic powder which contained 65 percent cacodylic acid (active ingredient), 30 percent sodium chloride, 3 percent sulfates and 2 percent water. Approximately 290 lb of powder were mixed with 100 gallons of water (6). Thus, a total of 5,200 gal of Blue were probably disseminated in South Vietnam (primarily by the HIDAL Spray System).

[c] Pink and Green contained approximately 8.16 lb active ingredient per gal (7).

[d] Purple contained approximately 8.14 lb active ingredient per gal (see Section V.A.3., Chapter I, p I-25)

TABLE 3. Estimated number of gal of military herbicide procured by the U. S. Department of Defense and disseminated in South Vietnam during the period January 1965 - February 1971.

Military Herbicide	Craig, 1974 (12)a	NAS Report, 1974 (11)b	Westing, 1976 (34)c
Orange	10,645,904	11,266,929	11,712,860
White	5,632,904	5,274,129	5,239,853
Blue	1,144,746	1,137,470	2,161,456
Total	17,423,554	18,936,068	19,114,169

[a] Data compiled from procurement and disposition records maintained by the San Antonio Air Logistics Center, Directorate of Energy Management, Kelly Air Force Base, Texas. The data for expenditures of Herbicide Orange, White, and Blue were based on procurement and delivery records for late FY 64 through FY 72, less those quantities of herbicides returned to Johnston Island in April 1972 or retained in the Continental United States.

[b] See Table III C-1 of the referenced National Academy of Science report (11).

[c] See Table 3.3 of the referenced report.

D. Land Area Sprayed with Herbicides in South Vietnam

As noted in Section C above, the estimate of acreage sprayed with herbicides was often based on spray line data and/or the quantity of herbicide expended. The National Academy of Science (11) discussed these parameters as they applied to the HERBS tape, the major source of all mission maps and tabulations of herbicide operations in South Vietnam for the period August 1965 through February 1971. Table 4 presents a comparison of data from three sources on the estimated number of acres treated in South Vietnam from January 1962 through February 1971. Included in these figures are the same areas of land counted more than once if they were sprayed more than once.

Table 5 is a comparison of the data for acreage sprayed within the three major vegetational categories. These data have been corrected for multiple coverage. The National Academy of Science Report (11) concluded that herbicides were sprayed on 10.3 percent of the inland forests of South Vietnam, 36.1 percent of the mangrove forests, and 3 percent of the cultivated lands or approximately 8.6 percent of the total land area in South Vietnam. Westing (34) estimated that approximately 10 percent of South Vietnam was sprayed.

III. THE AIRCRAFT, SPRAY SYSTEMS AND MISSION CONCEPT IN OPERATION RANCH HAND

Almost all herbicide used in South Vietnam was sprayed from aircraft. Irish et al (26) have described some ground delivery systems for herbicides, but noted these were used primarily for control of vegetation on minefields and perimeter defenses.

U.S. military personnel were responsible for operating and maintaining the aircraft used in Operation RANCH HAND. The number and types of aircraft, their load capacity, ease of loading, and the spray system employed in them, all were important factors in determining the number of personnel required in performing the herbicide missions. Standard procedures were adopted in all herbicide handling phases of the operation. This section, then, reviews the aircraft factors where military personnel were likely to have physically contacted the herbicides.

A. Historical

The first aerial spray trials for herbicides were conducted by the military in 1944 and 1945 (14, 26). These early tests were accomplished using the U.S Army Chemical Corps M-10 smoke tanks hanged externally on a B-25 aircraft. By 1953, the U.S. Air Force had accomplished prove-out and acceptance testing of the large-capacity (1,000-gal) spray system known as the Hourglass or MC-1 Spray System. In 1960 and 1961, Air Force personnel assigned to the Special Aerial Spray Flight, Langley AFB, Virginia, acquired two MC-1 Spray Systems

TABLE 4. Comparison of data from three sources of the estimated number of acres treated in South Vietnam during the period of January 1962 - February 1971. Data make no allowance for multiple coverage.

	ACRES TREATED			
YEAR	NAS Report (11)	Irish et al. (26)	Westing (34)	MEAN
1962	NA[a]	5,681	5,724	5,703
1963	NA	24,947	24,920	24,934
1964	NA	93,842	93,869	93,856
1965	75,501[b]	221,559	221,552	221,555
1966	608,106	842,764	845,263	765,378
1967	1,570,114	1,707,758	1,707,784	1,661,885
1968	1,365,479	1,330,836	1,696,337	1,464,217
1969	1,365,754	NA	1,519,606	1,442,680
1970	294,925	NA	252,989	273,982
1971	1,259	NA	3,346	2,303
			Total of Mean =	5,956,493

[a] Data not available (NA)

[b] Data for period August 65 through December 65.

TABLE 5. The number of acres treated in South Vietnam, 1962 - 1971, with military herbicides within the three major vegetational categories. Data represent areas receiving single or multiple coverage and for 90 percent of all areas treated.

Vegetational Category	ACRES TREATED NAS Report, 1974 (11)[a]	Westing, 1976 (34)[b]
Inland Forest	2,670,000	2,879,000
Mangrove Forest	318,000	746,000
Cultivated Crops	260,000	595,000
Total	3,248,000	4,221,000

[a] See page III-39 of the referenced report.

[b] See Table 3.6 of the referenced report, Data for Inland Forest was woody subtotal, less acreage for mangrove forest.

and modified them to spray insecticide and to interface with the newly acquired Fairchild-Hiller C-123 air transport.

Irish et al (26) noted that in October 1961, six C-123 aircraft were made available to the USAF Tactical Air Command with a high-priority directive to install the MC-1 Spray System. Fabrication was accomplished expeditiously and on 7 January 1962, three of the configured-aircraft arrived at Tan Son Nhut Air Base, Republic of Vietnam. During the early months of 1962, the C-123/MC-1 system and the HIDAL (Helicopter, Insecticide Dispersal Apparatus, Liquid) were evaluated for dissemination characteristics. The aircrews were members of the Special Aerial Spray Flight, Langley AFB, and were on temporary duty to South Vietnam as part of Operation RANCH HAND. Air Force personnel engaged in the herbicide program did not receive permanent change of station assignments until 1964.

In late 1962 and early 1963, an intensive RDT&E (Research Development, Testing and Evaluation) program was initiated between the Crops Division, Fort Detrick, and the Air Force Armament Laboratory, Eglin AFB, Florida, to provide improvements in spray system components in support of RANCH HAND (26). Concurrently, operational employment of the spray capability by the RANCH HAND units was intensified steadily with time and availability of resources.

B. *Spray Systems and Characteristics of the RANCH HAND Aircraft*

Tests and evaluations of aircraft and spray systems were conducted on the calibration grids on Test Area C-52A, Eglin AFB, Florida (6, 14, 22, 27, 35) and on the calibration grid at Pran Buri, Thailand (11, 13, 15).

The C-123/MC-1 spray configuration was initially calibrated to spray 1 to 1.5 gal of herbicide per acre (gal/A) (14, 26). Thus, in 1962 and 1963 the herbicide missions conducted using this initial system resulted in the dissemination of herbicide at this lower rate. The numerous modifications and extensive evaluations of the equipment configurations at Eglin AFB and Pran Buri did not result in equipment changes for Operation RANCH HAND until 1964 (14). Darrow et al (14) and Irish et al (26) have reported that in early 1964 the rate of 3 gal/A was obtained at first by making double passes with the aircraft but by late 1964 the modifications were complete and the system was capable of spraying 3 gal/A in a single pass. The modified 1,000-gal C-123/MC-1 spray system was capable of depositing 3 gal/A on swaths 240 feet wide when spraying at an airspeed of 130 knots at a 150 feet altitude. Two 20-hp pumps were needed to achieve the required flow rate of 430 gal/min of Purple (26).

The HIDAL spray system was capable of deposits of 1.5 gal/A when flown inwind at 55 knots and at an altitude of 100 feet (26). The tank volume for this system was 200 gal.

In early 1966, following its development, the A/A 45Y-1 Internal Defoliant Dispenser, replaced the MC-1 in all C-123 aircraft. However, completion of calibration tests and performance characteristics for this spray system did not occur until 1968 (16, 22, 27). The A/A 45Y-1 defoliant dispenser was a modular spray system for internal carriage in cargo aircraft. The module consisted of a 1,000 gal tank, pump, and engine (20 hp) mounted on a frame pallet. An operator's console was an integral part of the unit but was not mounted on the pallet. The C-123 aircraft had wing booms 1.5 inches in diameter and 22 feet long extending from the outboard engine nacelles toward the wing tips. A short tail boom 3 inches in diameter was positioned centrally near the aft cargo door. There were 16 nozzles on each wing boom and eight on the tail boom. The nozzles were check valve bodies with 3/8-inch orifices (no nozzle tips). The system was capable of spraying at the rate of 240 gal/min, which, when released at 150 feet altitude at 130 knots airspeed produced a swath 260±20 feet wide with a mean deposit of 3 gal/A in a coarse spray having an MMD (mass median diameter) of 320 to 350 micron (μ). Spraying time was approximately 3.5 to 4 minutes, which was adequate to dispense 950 gal of chemical on a spray line about 8.7 statute miles (14 km) in length. In order to achieve predictable deposits, it was recommended that the missions be conducted under inversion to neutral temperature situations and calm wind conditions. Craig (12) has reported that each aircraft had a crew of 3 men: the pilot, co-pilot (navigator), and flight engineer (console operator). However, observers (Vietnamese and American) frequently accompanied the aircrews on herbicide missions (32).

C. Mission Concepts

The objectives of the defoliation and anticrop programs in South Vietnam have been thoroughly reviewed by Huddle (23) and others (11, 14, 34). It is the objective of this section to elaborate only on the background and mechanics of a "typical" herbicide mission that would have influenced the degree of exposure to herbicides by aircrew and/or ground personnel. The following scenario of events or "standard operating procedures" has been compiled from reports by Craig (12), Darrow et al (14), Irish et al (26) and the National Academy of Science Report (11).

1. Each of the 11 different companies that manufactured military herbicides packed them in new ICC 17C 55-gal 18 gauge steel drums for shipment to Southeast Asia (12). Until 1967, lined drums were used only for shipment of Blue. However, because of the results of compatibility tests, lined drums were also used to ship White beginning in 1967.

2. Each herbicide drum was marked with a three-inch color-coded band around the center to identify the specific military herbicide. This marking was initially a 12-inch band, but was changed to a 3-inch band in March 1966.

3. Shipping time from the arrival of the herbicide at a U.S. port until it arrived in South Vietnam varied from 47 to 52 days.

4. About 10 out of every 10,000 drums shipped were received in a damaged or defective state. This represented a damage rate of 0.1 percent. About 50 percent of these damaged drums leaked as a result of punctures or split seams. These were caused by improper loading and defective drums. Forklifts operated by stevedores also caused punctures. Redrumming was accomplished at the ports.

5. About 65 percent of the herbicide was shipped to the 20th Ordnance Storage Depot, Saigon, and 35 percent was shipped to the 511th Ordnance Storage Depot, Da Nang. Under the normal handling procedures, drums were unloaded at Da Nang and Saigon from the cargo vessel directly into semi-trailers and were placed in an upright position. The trailers were driven to the various units of the 12th Air Commando Squadron (primarily at the bases of Da Nang, Phu Cat, or Bien Hoa) for disposition.

6. Normally the contents of the drums were transferred into blocked F-6 trailer tanks through a suction tube without removing the full drums from the semi-trailers. Each F-6 trailer held 4,298 gal or about 78 drums of herbicide. If blocked F-6 trailer tanks could not accommodate the total inventory, the drums were stacked in pyramidal style until needed.

7. The transfer of the herbicides from the 55-gal steel drums to storage tanks or aircraft tanks required some precautionary measures. Personnel charged with the supervisory responsibilities of handling the herbicides were indoctrinated in appropriate safety precautions including the use of gloves and face shields as needed. Personnel handling the chemicals were encouraged to "take normal sanitary precautions and to maintain personal cleanliness and to avoid skin and eye contact with the material. Contaminated clothing were to be washed before re-use. Spillage on the skin or in the eyes was to be rinsed copiously with clear water" (14).

8. When the herbicide was pumped from the drums into the F-6 trailers about 0.5 to 1.5 gal remained in the drum. Hence the drum was placed on a drain rack and the "drippings" were collected from many drums in a pan-type receptacle and used for spraying base perimeter areas.

9. Empty drums were given to the military forces (Vietnam, U.S. and Free World Military Assistance Forces) for use as barriers in defensive positions. The drums were filled with sand or concrete and used in the construction of bunkers or in foundations for runways and barbed wire perimeters (12).

10. Surface areas contaminated by spillage of the herbicides were flushed with diesel fuel or water with diversion of the drainage into settling basins or pits for incorporation into the soil.

11. The F-6 trailers were tied to plumbing and pumps so that the herbicide could be delivered to the aircraft without moving the trailers.

12. As previously noted, Orange was insoluble in water, while Blue and White were not. When Orange was mixed with either Blue or White, a gummy substance formed. The F-6 trailers were therefore color-coded to correspond to the drum color-codes and used exclusively for the herbicide to which the code applied.

13. The aircraft spray tanks, positioned in the center of the airplane, and the spray system were purged before the type of herbicide carried was changed. Particular attention had to be given to sequences involving Blue and White. A mixture of these two herbicides resulted in the formation of a precipitate consisting of the sodium salt of 2,4-D.

14. Most of the personnel involved in the actual handling of the herbicide drums were Vietnamese. However, a USAF flight mechanic or crew chief was responsible for insuring that the aircraft was properly loaded and the spray system functional. A flight mechanic was also the console operator for the spray unit. The pilot and co-pilot were officers while the flight mechanics and crew chiefs were usually enlisted personnel.

15. For record keeping purposes a herbicide "mission" consisted of several aircraft; if only one aircraft was used the operation was termed a sortie. All missions within a target formed a project.

16. Aircraft takeoffs were normally before sunrise. From a tactical point of view, the arrival of the aircraft at the target area just prior to sunrise permitted the aircraft to approach the target from the direction of the rising sun. This afforded some degree of protection from enemy ground fire. From the standpoint of herbicidal action, application by aerial spray was most effective if accomplished prior to 0800 hours while inversion conditions existed, in the absence of precipitation, and while the wind was calm or not exceeding a velocity of 8 knots. This insured the proper settling of the spray on the target area.

17. Within the aircraft, it was not uncommon to have herbicide leakage from around the numerous hose connections joining the spray tank and pumps with the wing and aft spray booms. In hot weather, the odor of herbicide within the aircraft was decidedly noticeable. Periodically, the spray tank and console were removed (especially with the portable A/A 45Y-1 system) and the interior flushed with surfactant or soap and with water. Because of the corrosive nature of some herbicides, it was necessary for the aircraft to also be repainted periodically.

18. In the 1966 through 1968 period, more than one sortie per day was often common. For example, during the first six months of 1968, the 24 UC-123B aircraft assigned to RANCH HAND averaged approximately 39 sorties per day.

IV. PERTINENT DEPLOYMENT AND BIOLOGICAL FACTORS OF THE HERBICIDES

The previous section dealt with those factors that would influence the frequency of "physical contact" with liquid forms of the herbicide. As noted, the individuals most likely to be exposed to liquid herbicide were those charged with transport, handling, and disseminating responsibilities. This section will deal with some factors that would have influenced the likelihood of contact with the herbicides once they had been sprayed.

A. Use Patterns of Individual Military Herbicides

1. Herbicides Orange, Orange II, Purple, Pink and Green

Herbicides Orange, Orange II, Purple, Pink and Green were effective defoliants and herbicides on a wide array of woody and broadleaf herbaceous species. Grasses, bamboos, and other monocotyledonous plants were less affected. The effects of these military herbicides on the forests of South Vietnam has been well documented (5, 9, 11, 13, 14, 21, 29, 32, 34). Darrow (13), and Darrow et al (14, 15) showed that at the normal use rates (3 gal/A) these herbicides, when applied to mixed woody vegetation, caused a browning and discoloration of the foliage within a period of one or two weeks. Foliage of the more susceptible species turned brown rapidly, and subsequent leaf drop occurred over a period of one to two months. Under tropical conditions, maximum defoliation occurred two to three months after the spray application. At 3 gal/A the maximum average defoliation in a single or multiple canopy was 88 and 75 percent respectively for rainy season application, or 82 and 67 percent respectively for dry season application. Under tropical forest conditions, satisfactory levels of defoliation persisted for four to twelve months or more. The National Academy of Science (11) reported that from August 1965 through February 1971, 2,962 herbicide missions (out of a total of 6,237 missions for all herbicides and all uses) were for forest defoliation using Orange. These 2,962 missions accounted for 90 percent of all Herbicide Orange (including Orange II) used in South Vietnam. Likewise 90 percent of all Purple, Pink and Green sprayed in South Vietnam was for forest defoliation (26). Orange and Orange II (and Purple) were also used in control of broadleaf crops (8, 11, 34). For convenience and simplification in programming crop destruction and defoliation targets, application rates were routinely 3 gal/A (14). Annual crops; e.g., beans, gourd, jute, peanuts, and ramie, were rapidly killed by an application of Orange. Root or tuber crops; e.g., manioc, potatoes, taro, and yams, showed great reduction in yield when treated with Orange during early growth stages. Perennial and woody tropical crops; e.g., jackfruit, papaya, castor bean, and mango were susceptible to Herbicide Orange (14). From August 1965 through February 1971, crop destruction missions with Orange accounted for 8 percent of the Herbicide Orange applied (11).

The remaining 2 percent of Herbicide Orange used in South Vietnam was used around base perimeters, cache sites, waterways, and communication lines (11).

2. Herbicide White

Herbicide White was effective principally on broadleaf herbaceous and woody plants. Conifers (pine trees) were especially susceptible to White. However, the herbicidal action on woody plants was slow and full defoliation did not occur for several months after spray application (14). Since White was water soluble, it was frequently used in field situations where drift was to be held at a minimum (e.g., near rubber plantations). White was sprayed during 1,324 defoliation missions (21 percent of all missions) and of the total volume of White used in South Vietnam, 99 percent was for defoliation (11). The remaining one percent of White was used primarily in base perimeter applications. White was not recommended for use on crops because of the persistence of picloram in soils (14).

3. Herbicide Blue

Herbicide Blue was the herbicide of choice for other crop destruction missions; e.g., on cereal or grain crops. For crop destruction missions, the basic rate of 3 gal/A was used. Helicopter applications were usually at the rate of one gal/A for control of grain crops. (15). Forty-nine percent of all Blue (580,000 gal) was used in crop destruction missions conducted from August 1965 through February 1971 (11). The remaining Blue was used in defoliation or in control of grass around base perimeters (11). As a defoliant, Blue caused a rapid browning or desiccation with accompanying shriveling and leaf fall. Noticeable browning or discoloration was evident in one day, with maximum defoliation occurring within two to four weeks (14).

B. Canopy Penetration of Defoliants

As previously noted, 90 percent of all Herbicide Orange (and probably Purple, Pink and Green) was for defoliation in the forests and mangroves of South Vietnam. The quantity of herbicide that reached the forest floor is not known. However, such factors as canopy composition and time of season of application would have influenced this value.

Huddle (23) recorded the following 1968 statement by Dr C.E. Minarik (Dr Minarik was at that time Director, Plant Sciences Laboratories, Fort Detrick, Maryland):

> "Three gallons per acre is employed. We would prefer to use less if we could get uniform deposition, but in these dense jungle areas where there may be 300 tons of vegetation per acre, this is the minimal effective volume. The three gallons contain 24 pounds of herbicide on an acid basis. Thus high dosage rate is also a requirement since much of the vegetation consists of trees 100 to 150 feet tall."

In the evaluation tests of the C-123/A/A 45Y-1 Spray System, Harrigan (22) and Klein and Harrigan (27) found that in mass distribution studies (following aerial dissemination) 87 percent of the Orange Herbicide intercepted by collecting devices had a mass median diameter between 100 and 500μ. The mean diameter was 367μ. Harrigan (22) concluded that with altitude delivery conditions at 130 knots and 150 feet altitude, most of the Orange released would have settled onto the forest canopy in a swath approximately 260±20 feet wide within which effective defoliation was produced. Hurtt and Darrow (25) showed that the minimum biological effective deposition rate under the climatic conditions of South Vietnam was 1.0 gal/A at a mass median diameter of 350μ. The minimum biological effective rate was defined as "that rate which promoted leaf fall and inhibited growth" (25).

In canopy penetration studies, Tschirley (33) found (with phenoxy herbicide formulations similar to Orange) that the volume of spray reaching lower sampling levels varied proportionately with the amount deposited on the top line above the canopy. On the average, about 21 percent of the spray penetrated the upper canopy and about 6 percent penetrated to ground level. He also found that the percentage penetration remained relatively constant for drop densities greater than about 100 per square inch. Spray drops having mass median diameters of 400 to 500μ would approximately equal 100 drops per square inch. Moreover, the percent spray penetration through forest canopies was inversely related to canopy density (33).

No data were available on the number of defoliation missions conducted during the wet or dry seasons. However, as noted earlier, defoliation of forest canopy was greatest during the rainy season, when the vegetation was in full-leaf and actively growing.

V. ESTIMATED QUANTITIES OF INDIVIDUAL CHEMICALS SPRAYED IN SOUTH VIETNAM

For this report, the total quantities of individual chemicals become important only in reference to their potential association with dose and duration of exposure to the population at risk. Although the National Academy of Science (11) primarily defined the population at risk as Vietnamese (especially Montagnards), our concern at this time is with U.S. military forces.

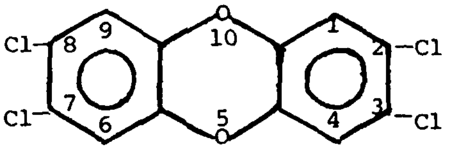
The chemicals of concern are 2,4-D, 2,4,5-T and TCDD. The extreme toxicity of TCDD, however, makes it the prime chemical of concern. The toxicology of these chemicals is discussed in detail in Chapters IV and VI. The previous scientific assessments of these chemicals as applied in South Vietnam are addressed in Chapter V.

A. Herbicide Orange and its Components 2,4-D, 2,4,5-T and TCDD

1. Concentrations of TCDD in Orange, Purple, Pink and Green

Figure 2 shows the structure of TCDD and gives a brief description of some of its physical and chemical characteristics. Table 6 shows the available data on the concentration of TCDD (parts per million, ppm) in samples of Herbicides Orange and Purple. As noted, the mean concentration of the surplus Herbicide Orange remaining after termination of its use in South Vietnam was a value derived from data on the analyses of 492 samples. Some of these data have been previously published (4, 11). Craig (12) has reported that the Orange Herbicide maintained at the Naval Construction Battalion Center (NCBC), Gulfport, Mississippi, was probably authorized and procured during the 1968-69 fiscal year. The Orange returned from Vietnam in 1972 (to Johnston Island) was procured no earlier than late FY 64, since the first shipment of Orange did not arrive in Vietnam until early 1965, and a six months lead time was typical. Note that the mean TCDD concentration was 1.91 ppm for the Johnston Island inventory and weighted means of 1.77 and 2.11 for samples analyzed from the NCBC inventory. It is important to note the range of TCDD concentration of TCDD in both surplus Orange inventories. The maximum concentration of TCDD in Orange samples collected at NCBC was 15 ppm, while the maximum concentration of TCDD reported in samples from Johnston Island was 47 ppm. Only 4 of 200 samples from Johnston Island exceeded TCDD levels found in the NCBC inventory (4). The values of these 4 samples were 17, 22, 33, and 47 ppm (4).

A. Structure



2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD)

B. Physical Characteristics

molecular weight	322
melting point, °C	303 - 305
decomposition point, °C	980 - 1,000

C. Chemical Characteristics

Solubility, grams/liter

ortho-dichlorobenzene	1.40
chlorobenzene	0.72
Orange Herbicide	0.58
benzene	0.57
chloroform	0.37
acetone	0.11
normal-octanol	0.05
lard oil	0.04
methanol	0.01
water	2×10^{-7}

FIGURE 2. Structure and physical/chemical characteristics of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin, TCDD or dioxin.

TABLE 6. Concentration, ppm, of TCDD in samples of Herbicides Orange and Purple.[a]

Source of Samples	Number of Samples		Range of TCDD (ppm)	Mean TCDD, Concentration (ppm)
	Orange	Purple		
Johnston Island Inventory, 1972[b]	200	(4)[c]	0 05-47	1.91
Johnston Island Inventory, 1974	10		0.07-5.3	1.68
NCBC, Gulfport Inventory, 1972[d]	42		0.05-13.3	1.77
NCBC, Gulfport Inventory, 1975	238		0.02-15	2.11
Eglin AFB Archived Sample		1[e]	-	45
Eglin AFB Inventory, 1972	2		-	0.04

The Weighted Mean Concentration of TCDD in Orange = 1.98 ppm

[a] Analyses for TCDD performed by Interpretive Analytical Services, Dow Chemical U.S.A., Midland Michigan; Aerospace Research Laboratories, Wright-Patterson AFB, Ohio; and The Brehm Laboratory, Wright State University, Dayton Ohio.

[b] Surplus Herbicide Orange was shipped from South Vietnam to Johnston Island for storage in April 1972.

[c] Four of 200 samples may have been Herbicide Purple, see text.

[d] The Naval Construction Battalion Center (NCBC) Gulfport, Missippi served as a storage site for Surplus Herbicide Orange from 1969 to 1977.

[e] Herbicide Purple was extensively used in the evaluation of aerial spray equipment on Test Area C-52, Eglin Air Force Base Reservation, Florida, 1962-1964.

Only one sample of Herbicide Purple has been analyzed (see Table 6). The age of the sample was not known except that it was representative of the Purple applied to Grid 1 (ARPA Grid), Test Area C-52A, Eglin AFB, Florida (Personal information, A.L. Young, and references 34 and 36), and thus, may have been from the 1962-1964 time period. The 1971 Report on 2,4,5-T by the Executive Office of the President (18) presented data on TCDD concentrations found in the analysis of Technical 2,4,5-T from one manufacturer. The data were for samples manufactured yearly from 1958 through 1969, and ranged from 1 to 32 ppm. The highest levels of TCDD were found in samples manufactured in 1965 (32 ppm) and 1968 (25 ppm). *If these two samples* had been used in formulating Herbicide Orange, the concentrations in the Orange would have been 16 and 12.5 ppm, respectively. *If the lowest* TCDD containing samples for the same two years would have been used (i.e., samples containing 5 and 1 ppm TCDD) the concentrations in the Orange would have been 2.5 and 0.5 ppm, respectively. The one sample of Purple reported in Table 6 contained 45 ppm. Thus, the Technical 2,4,5-T used in that sample may have contained 90 ppm TCDD.

When the Orange Herbicide was shipped to Johnston Island from South Vietnam, redrumming of the herbicide in South Vietnam was accomplished as necessary (12). The project (PACER IVY) involved U.S. military personnel. One of the individuals participating in the redrumming operation at Da Nang (redrumming also occurred at Phu Cat and Bien Hoa) has stated that drums of Purple were found (although fewer than 20) and redrummed into Orange-banded drums (personal communication, Dr Michael D. Neptune, now with the U.S. Environmental Protection Agency, Washington, D.C.). In addition, an analytical chemist involved in the analyses of Orange samples for 2,4-D and 2,4,5-T, reported finding significant quantities (15 percent) of the iso-butyl ester 2,4,5-T in a few of the samples collected from Johnston Island (unpublished data, personal communication, Dr Eugene L. Arnold, now with the Clinical Sciences Division, USAF School of Aerospace Medicine, Brooks AFB, Texas). Thus, the 4 samples of Orange Herbicide containing TCDD concentrations greater than 15 ppm, may have been Purple. If these were, in fact, from drums of Purple, then the mean concentration of TCDD in 5 samples of Purple would have been 32.8 ppm.

The mean value of 32.8 ppm may or may not represent the TCDD concentration of the Herbicide Purple used in South Vietnam from 1962 through 1964. Data from the 1971 Report on 2,4,5-T (18) suggests that Purple manufactured in 1958 through 1963 would have had a mean concentration of approximately 5 ppm (4.7±1.2 as the mean and standard deviation for the 6 samples reported for the years 1958 through 1963). However, the persistence of TCDD in soils of the two grids used for the testing and evaluation of the early RANCH HAND spray systems may indicate that Purple indeed had concentrations of TCDD from 17 to 47 ppm. Young (35) and Young et al (37) reported finding concentrations of 710 parts per trillion TCDD in the top 6 inches (15 cm) of soil collected in 1974 from the equipment-testing grid known to have received at least 1,894 lb of Purple per acre during the 1962 through 1964 period. The test grid had received 16,164 gal of Purple. On an adjacent test grid, 1,168 pounds of Orange per acre had been disseminated during the 1964-1966 programs evaluating the A/A 45Y-1 Spray System. The levels of TCDD in the soil treated with Orange at comparable depth was 30 parts per trillion. All soil samples were analyzed in 1973. Young et al (36) have reported the half-life of TCDD to be less than one year when in the presence of the phenoxy herbicides. Persistence data suggested that the levels of TCDD in Purple and Orange were significantly different. Further evidence of this is recorded by the National Academy of Science (11) for TCDD residue found in the soils of the Pran Buri Calibration Grid. They reported finding levels from <0.0012 to 0.233 ppm TCDD in the top 6 inches of soil from this testing ground. They concluded that since the grid had received approximatly 1,000 lb/A 2,4,5-T in 1964-65, the original concentration of the TCDD in Orange would have ranged from <3 to 50 ppm. The NAS Committee (11) assumed that the material applied to the Pran Buri Calibration Grid was Orange. Darrow et al (15), responsible for the original calibration studies, reported that the Pran Buri Calibration Grid received 6,000 gal Purple, 3,800 gal Pink (all 2,4,5-T) and only 825 gal Orange. Since the majority of herbicide applied on this grid was either Purple or Pink, it further supports the contention that the four high-TCDD-containing samples from Johnston Island were Purple.

Accepting the mean concentration of TCDD in Purple as 32.8 ppm and recognizing that Pink and Green contained essentially twice the active ingredient (8.16 lb acid equivalent 2,4,5-T per gal) as Purple (4.0 lb acid equivalent 2,4,5-T per gal), the mean concentration of TCDD in Pink and Green would have been twice that of Purple, or 65.6 ppm.

Also, from the above discussion, it can be concluded with reasonable certainty that the weighted mean concentration for all Herbicide Orange sprayed in South Vietnam was 1.98 ppm: individual lots may have contained higher (≤15 ppm) or lower (≥ 0.02 ppm) concentrations of TCDD, but the weighted mean was 1.98 ppm.

2. Concentrations of 2,4-D and 2,4,5-T in Orange

The original military specifications for Herbicide Orange were published on 19 July 1963 as specifications MIL-H-51158 (MU) and MIL-H-51147 (MU). As written in the specifications, for the n-butyl ester of 2,4-D: "The total acid equivalent of the herbicide shall be not less than 78 nor more than 80 percent when tested as specified. The free acid content of the herbicide shall not be greater than 1.0 percent." For the n-butyl ester of 2,4,5-T the specifications noted: "The total acid equivalent of the herbicide shall be not less than 80 nor more than 82 percent when tested as specified. The free acid content of the herbicide shall not be greater than 1.0 percent." Orange was to be a 50:50 mixture of the products from the two specifications. These specifications were updated on 7 November 1966.

Fee et al (20) and Hughes et al (24) have extensively analyzed Herbicide Orange samples (from Johnston Island and NCBC, Gulfport, Mississippi) for composition. Table 7 is a comparison of different manufacturers' lots for percent composition. Although the actual mean composition varied from the "theoretical" specification, the analytical method employed to test the total acid equivalent of the herbicide permitted some fluctuation in content.

The parent acid portion of the herbicide molecule will remain as the herbicidally active portion of its respective ester form, while the ester appendage to the parent acid form will serve to satisfy some additional properties, such as decreased water solubility and increased surface penetration and/or translocation. The butyl ester of 2,4-D contained 79.4 percent acid 2,4-D and the butyl ester of 2,4,5-T contained 80.2 percent acid 2,4,5-T. From the data in Table 7, the mean actual weight of active ingredient per gal of Orange was 4.14 and 4.00 pounds for 2,4-D and 2,4,5-T, respectively. These values have been accepted also for the active ingredients in Herbicide Purple.

3. Quantities of Herbicides and TCDD Disseminated in South Vietnam

Using data in Tables 2 and 3 (herbicide procurement records), Table 6 (mean TCDD concentration in Orange) the value of 32.8 ppm TCDD for Purple,the value of 65.6 ppm TCDD for Pink and Green, and Table 7 (mean, actual composition of Herbicide Orange), an estimate of the quantities of herbicides and TCDD disseminated in South Vietnam from January 1962 through February 1971, can be determined. These "estimated" quantities are in Table 8. The National Academy of Science Committee on the Effects of Herbicides in South Vietnam (11) estimated that between 220 and 360 pounds of TCDD were released over South Vietnam during the period August 1965 to February 1971. The estimate of 368 pounds in Table 8 for TCDD falls very close to their estimate. The important difference is that 143 pounds of the TCDD reported in Table 8 (or approximately thirty-nine percent of all the TCDD) was contained in Purple, Pink, and Green and was sprayed on 90,000 acres in Vietnam from 1962 through 1964, a time period when only a small force of military personnel were in South Vietnam. Herbicide Orange was sprayed on 3.5 million acres from 1965 through 1970. However, 90 percent of the Orange was sprayed on 2.9 million acres of inland forests and mangrove forests.

TABLE 7. Composition, Percent, of Selected Samples of Herbicide Orange in Relation to Military Specifications.

	NCBC Inventory Number[a]				
Component	ASN 8	ASN 10	ASN 14	Mean Composition	Approximate Military Specification[b]
Number of Gallons	123,695	383,955	145,860		
Level of TCDD	<0.02 ppm	0.30 ±0.06 ppm	<0.02 ppm		
n-Butyl ester 2,4-D	42.6%	46.2%	43.7%	44.2%	49.5%
n-Butyl ester 2,4,5-T	39.3	44.9	42.2	42.1	48.8
Other Butyl esters of chlorophenoxyacetic acids	7.96	4.01	9.05	7.0	-
Octyl esters of chlorophenoxyacetic acids	5.76	0.25	-	2.0	-
Acid, 2,4-D	0.78	0.19	0.65	0.5	0.1
Acid, 2,4,5-T	0.84	0.13	0.78	0.6	1.0
Inert Ingredients[c]	2.76	4.32	3.62	3.6	0.6

[a] Selected samples of Herbicide Orange were collected from the surplus inventory maintained at the Naval Construction Battalion Center (NCBC), Gulfport, Mississippi. Samples represented lots produced by different manufacturers. Analyses for TCDD and sample composition were performed by the Aerospace Research Laboratories, Wright-Patterson AFB, Ohio. [See Reference by Hughes et al. (24).]

[b] Military specifications for manufacture of Herbicide Orange were based on Specifications MIL-H-51147A (MU) and MIL-H-51148A (MU) dated 7 Nov 1966.

[c] Inert ingredients included butanol, toluene, butylchloride, dichlorophenol, trichlorophenol, butoxydichlorobenzene, and butoxytrichlorobenzene.

TABLE 8. Estimated quantities of herbicides and TCDD disseminated in South Vietnam from January 1962 - February 1971.

Chemical	Pounds
2,45-D[a]	55,940,150
2,4,5-T[b]	44,232,600
TCDD[c]	368
Picloram[d]	3,041,800
Cacodylic Acid[e]	3,548,710
Total of Herbicides	106,763,260

[a] 2,4-D was an active ingredient in Herbicides Orange, Purple and White. From data in Table 7, the acid equivalents for 2,4-D in Herbicide Orange and White were calculated to be 4.14 lb/gal and 2.00 lb/gal, respectively. The acid equivalent for 2,4-D in Herbicide Purple was assumed to be 4.14 lb/gal.

[b] 2,4,5-T was an active ingredient in Green, Pink, Purple and Orange. Approximately 276,000 gal of Green, Pink and Purple were sprayed in South Vietnam prior to 1965, when it was replaced by Herbicide Orange. Herbicides Green and Pink contained 8.16 lb/gal 2,4,5-T. Herbicides Purple and Orange contained 4.00 lb/gal 2,4,5-T (Table 7).

[c] The mean TCDD concentration in Herbicide Purple was estimated at 32.8 ppm. The mean TCDD concentration in Herbicides Pink and Green was estimated at 65.6 ppm. The mean TCDD concentration in Herbicide Orange was estimated at 1.98 ppm.

[d] Picloram was an active ingredient of Herbicide White.

[e] Cacodylic acid was the acitve ingredient of Herbicide Blue. The Herbicide Blue formulation contained 15.4 percent arsenic in the pentavalent organic form. The value includes 10,000 lb cacodylic acid disseminated in South Vietnam from 1962-1964.

B. Military Projects that Involved Handling Herbicides Orange, Purple, Pink or Green.

Herbicide Orange was first manufactured in late 1964. It arrived in Vietnam for use in Operation RANCH HAND in early 1965. Prior to Orange, Herbicides Purple, Pink and Green were used but in far less quantities and on a limited area. All of the quantities of Orange returned from Johnston Island in 1972 and those stored at the Naval Construction Battalion Center since late 1968 were destroyed by at-sea incineration in 1977.

From the first aerial spray test in 1961 through the incineration project in 1977, numerous U.S personnel directly handled the herbicide in support of specific project goals. Table 9 was assembled after an extensive search of available documents and from personal contact with eleven different individuals that had participated in one or more of the listed projects.

Other than Operation RANCH HAND the most extensive handling of Herbicide Orange occurred during Project PACER HO. At the time of this latter project, analytical techniques were sufficiently developed to permit the environmental monitoring of TCDD at the parts per trillion level during all stages of the project. These data permitted an assessment of the actual exposure of personnel involved in the handling of the herbicide. Chapter II is devoted to Project PACER HO, the disposal of the surplus Herbicide Orange.

VI. SUMMARY

The choice of herbicides used in South Vietnam in Operation RANCH HAND, 1962-1971, was based upon those herbicides that had been widely used in world agriculture, shown to be effective in controlling a broad specturm of vegetation, and proven safe to humans and animals. The major herbicides used in South Vietnam were the phenoxy herbicides 2,4-D and 2,4,5-T. These two herbicides were formulated as the water insoluble esters and code-named by the military as Purple, Orange, Pink and Green. A water soluble amine formulation of 2,4-D was used in Herbicide White. Two other herbicides were extensively used by the military, picloram (in White) and cacodylic acid (in Blue).

An estimated 107 million pounds of herbicides were aerially-disseminated on 6 million acres in South Vietnam from January 1962 through February 1971. Approximately 94 percent of all herbicides sprayed in Vietnam were 2,4-D (56 million pounds or 53 percent of total) or 2,4,5-T (44 million pounds or 41 percent of total). The 44 million pounds of 2,4,5-T contained an estimated 368 lb of the toxic contaminant, 2,3,7,8-tetrachlorodibenzo-*p*dioxin (TCDD or dioxin). Ninety-six percent of all 2,4,5-T was contained in Herbicide Orange; the remaining 4 percent in Herbicides Green, Pink and Purple. However, Herbicides Green, Pink and Purple contained approximately 40 percent of the estimated amount of TCDD disseminated in South Vietnam.

TABLE 9. Data on the major military projects involved in the handling and/or spraying of Herbicides Orange, Purple, Pink or Green in support of military programs in South Vietnam.

Project	Dates	Brief Description	Selected References
Project AGILE	1960-1968	Selection of herbicides, and development and evaluation of defoliation techniques.	Brown, 1962 (7) Coates et al, 1962 (10) Darrow et al, 1966 (15) Demaree and Creager, 1968 (16)
Operation RANCH HAND	1962-1971	Aerial spraying of herbicides in South Vietnam.	Anonymous, 1961 (3) Fair, 1963 (19) Ellison, 1967 (17) Darrow et al, 1969 (14) Huddle, 1969 (23) McConnell, 1970 (29)
USAF Projects 2525, 5172 5186, 5957	1962-1970	Development and testing of aerial spray equipment	Biever, 1969 (6) Klein and Harrigan, 1969 (27) Harrigan, 1970 (22)
Project PACER IVY	1971	Redrumming and movement of surplus herbicide from South Vietnam to Johnston Island	Craig, 1975 (12)
AFLC Project on Disposition of Herbicide Orange	1972-1977	Maintenance of herbicide inventory and research on options for disposal	Young, 1974 (35) Anonymous, 1974 (4) Lavergne, 1974 (28) Newton, 1975 (30) Young, et al, 1976 (37)
Project PACER HO	1977	Dedrumming of herbicide inventory and at-sea incineration of Herbicide Orange	Ackerman et al, 1978 (1)

Green, Pink and Purple were sprayed as defoliants on less than 90,000 acres from 1962 through 1964, a period when only a small force of U.S. military personnel were in South Vietnam. Ninety percent of all the Herbicide Orange (containing 38.3 million pounds of 2,4,5-T and 203 lb of TCDD) were used in defoliation operations on 2.9 million acres of inland forests and mangrove forests of South Vietnam.

The handling, transport and storage procedures employed for the herbicide generally precluded physical contact with the herbicides by most military personnel assigned to Operation RANCH HAND. However, flight mechanics (console operators for the internal spray systems) and crew chiefs (responsible for loading the aircraft) were the most likely military personnel exposed to the herbicides.

The methods employed in spraying the herbicides and the geographical areas designated for dissemination of the herbicides generally precluded direct physical contact with the herbicide by military personnel assigned to other military programs.

CHAPTER I
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CHAPTER II

DISPOSAL OF HERBICIDE ORANGE

I. INTRODUCTION

During the summer of 1977 the United States Air Force (USAF) disposed of 2.22 million gallons (gal) of Herbicide Orange by high temperature incineration at sea. This operation, Project PACER HO, was accomplished under very stringent criteria of U.S. Environmental Protection Agency (EPA) ocean dumping permits. Numerous conditions of these EPA permits required the USAF to conduct extensive environmental and occupational monitoring of the land-transfer/loading operations and shipboard incineration operations. The results of EPA permit compliance monitoring for ship board operations are reported elsewhere (1). The purpose of this chapter is to summarize the historical background leading to Project PACER HO, to briefly describe the land-transfer operations and to present a summary of industrial hygiene and ambient air monitoring accomplished during the land-based operations. At the time of this writing not all occupational and environmental monitoring data are available; thus, the final reports of land-based monitoring for project PACER HO have not yet been published.

II. HISTORICAL BACKGROUND

In April 1970, the Secretaries of Agriculture; Health, Education and Welfare, and the Interior jointly announced the suspension of certain uses of 2,4,5-T. These suspensions resulted from published studies indicating that 2,4,5-T was a teratogen. Subsequent studies revealed that the teratogenic effects had resulted from a toxic contaminant in the 2,4,5-T, identified as 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD). Subsequently, the Department of Defense suspended the use of Herbicide Orange (3). At the time of the suspension, the Air Force had an inventory of 1.37 million gal of Herbicide Orange in South Vietnam and 0.85 million gal at the Naval Construction Battalion Center (NCBC) Gulfport Mississippi. In September 1971, the Department of Defense directed that the Herbicide Orange in South Vietnam be returned to the United States and that the entire 2.22 million gal be disposed of in an environmentally safe and efficient manner. The 1.37 million gal were moved from South Vietnam to Johnston Island, Pacific Ocean, for storage (Project PACER IVY) in April 1972. The average concentration of TCDD in the Herbicide Orange was about 2 parts per million and the total amount of TCDD in the entire Herbicide Orange stock was approximately 44.1 pounds.

Various techniques of destruction and recovery of the herbicide were investigated from 1971 to 1974 (AFLC Project on Disposition of Herbicide Orange). Destructive techniques included soil biodegradation, high temperature incineration, deep well injection, burial in underground nuclear test cavities, sludge burial and microbial reduction. Techniques to recover a useful product included use, return to manufacturers, fractionation and chlorinolysis.

Of these techniques, only high temperature incineration was sufficiently developed to warrant further investigation. The other methods were rejected because of several considerations, including long lead times for development, inadequate assurance of success, and the lack of industrial interest.

In December 1974, the USAF filed a final environmental impact statement (3) with the President's Council on Environmental Quality on the disposition of Herbicide Orange by destruction aboard a specially designed incineration vessel in a remote area of the Pacific Ocean, west of Johnston Island.

The EPA held a public meeting in February 1975 to consider an ocean incineration permit application submitted by the USAF in accordance with the Marine Protection, Research and Sanctuaries Act of 1972 as amended, 33 U.S.C. 1401 et seq. During this meeting, testimony was presented which indicated that techniques for chemically reprocessing the herbicide to remove unacceptable quantities of TCDD might have been developed. The EPA indicated that the option for reprocessing should be further explored as a means of disposition prior to making a decision to destroy the herbicide via incineration (7).

Subsequently, the USAF undertook an investigation into the feasibility of reprocessing Herbicide Orange. Pilot plant studies were conducted from the fall of 1975 to July 1976 on selective activated carbon adsorption of TCDD from herbicide. This reprocessing method was shown to be technically and environmentally feasible; however, a feasible and environmentally acceptable method of safely disposing of the TCDD-laden activated carbon was not demonstrated. The USAF concluded in February 1977 that the option of reprocessing was not feasible, timely or cost effective since a technique for the ultimate disposal of the activated carbon was not currently available or anticipated in the foreseeable future.

Consequently, on 9 March 1977, the USAF requested reconvening the EPA public hearings. As a result of the public hearing held on 7 April 1977, the EPA issued a research permit to the USAF and Ocean Combustion Services, B.V. (OCS) (6). This permit authorized the transport of the Herbicide Orange from the Naval Construction Battalion Center, Gulfport MS to a designated site in the North Pacific Ocean for the purpose of at-sea incineration in accordance with the provisions of the Marine Protection, Research and Sanctuaries Act of 1972, as amended. The vessel contracted for the at-sea incineration was the Dutch-owned ship, M/T Vulcanus, a ship registered in Singapore and previously used in the North Atlantic Ocean and the Gulf of Mexico to destroy chlorinated hydrocarbon wastes (12). A total of three herbicide loadings were required to incinerate the total stocks of Herbicide Orange: one loading from Gulfport MS and two loadings from Johnston Island.

III. DESCRIPTION OF LAND-BASED OPERATIONS

The operations at both storage sites were similar in many ways. At both sites, the 55-gal drums of Herbicide Orange were transported short distances from their storage location to a centralized facility. The herbicide was drained from the drums and transferred to the M/T Vulcanus. Following emptying, the drums were rinsed with diesel fuel, and subsequently crushed. The rinsing from empty drum cleaning was combined with the herbicide and transferred to the ship for later incineration at sea.

A. NCBC, Gulfport MS

The centralized dedrumming facility at the NCBC was a temporary, enclosed facility measuring approximately 35 feet by 35 feet with an interior ceiling height of approximately 10 feet. A ventilation system capable of providing approximately 57 air changes per hour was equipped with in-line activated charcoal filters to reduce vapor emissions to the outside air. Within this enclosed facility were four identical processing lines. Each line consisted of a self-closing entry door to admit full drums, a roller conveyor along which drums were moved in an upright position, a position equipped with a heavy duty electrically operated deheading cutter, a suction wand to remove the greatest portion of the herbicide from a deheaded drum, a spray device beneath the conveyor over which the deheaded and emptied drum was inverted and rinsed with two gal of diesel fuel, a commercial, heavy duty drum crusher and a self-closing exit door through which the crushed drums were passed.

Once each drum was deheaded the contents were removed by the suction wand, leaving approximately three gal of liquid in the drum. The drum was then manually inverted and the remaining herbicide was collected in an open trough beneath the conveyor. Each drum was permitted to drain into the same trough for a minimum period of five minutes after which it was sprayed with two gal of diesel fuel, allowed to drain while still inverted for a minimum of two minutes, and then crushed end-to-end to approximately one-third its original volume. The rinsed and crushed drum was passed through the exit door and stacked with all other crushed drums.

The liquid herbicide from the suction wands, and the herbicide and diesel fuel rinsing from the below-grade, open trough were pumped to 10,000 gal capacity rail tank cars. Air displaced from the tank cars during filling was filtered through an activated charcoal filter. The rail cars were moved along a rail spur approximately two miles to a dockside location where the herbicide was transferred to the incinerator ship, M/T Vulcanus. Displaced air from the ship's cargo tanks was also filtered through activated charcoal.

A total of 15,480 drums of Herbicide Orange was processed in this fashion at the NCBC between 24 May 1977 and 10 June 1977. Two 8-hour shifts of approximately 55 men each accomplished the dedrumming/transfer operations. These men were all USAF officers/technicians from the five Air Logistics Center of the Air Force Logistics Command located at Kelly AFB, Texas; Hill AFB, Utah; Robins AFB, Georgia; Tinker AFB, Oklahoma and McClellan AFB, California. All workers were provided daily changes of freshly laundered work clothes and men working within the dedrum facility were provided protective clothing including cartridge respirators, face shields, rubber aprons and rubber gloves. With only few exceptions the men rotated through all jobs involved in the dedrumming/transfer operations. All personnel were given pre-operational and post-operational physical examinations consisting of a complete medical history, complete neurological examination and the following laboratory procedures:

1. Complete hemoglobin, including hematocrit and platelet count
2. Prothombin time
3. Serum lipids
4. Serum glutamic oxaloacetic transaminase (SGOT) or
5. Serum glutamic pyruvate transaminase (SGPT)
6. Serum bilirubin
7. Blood glucose
8. Complete urinalysis
9. Chest x-ray

B. Johnston Island

The centralized dedrum facility at Johnston Island was a temporary, open facility measuring approximately 30 feet by 90 feet consisting of a concrete pad, roof and moveable canvas walls to exlude rain. This open facility was located adjacent to the Herbicide Orange storage site on the northwest end of Johnston Island. Nearly constant east winds ranging from 10 to 20 miles per hour provided natural ventilation and carried released vapors away from occupied areas. Two processing lines consisting of fabricated metal racks and open troughs were located in the west two-thirds of the facility. The east one-third contained pumps and drive-through for fuel trucks that were used to transport the dedrummed herbicide to the M/T Vulcanus. Full drums of herbicide were transported to the dedrum facility in sets of four using forklifts equipped with specially designed clamps. The drums were placed on the inclined metal racks in four groups of 12 drums each. Each set of 12 drums was handled independently by the dedrumming crew. Once a set of 12 drums was on the rack and the forklifts had withdrawn, a crew member would punch one hole near the top of each inclined drum as a vent hole to allow the crew's supervisory personnel to check the contents. Any drums containing other than Herbicide Orange were removed from the line and held for further testing. Three or more closely spaced holes were then punched in the bottom of each drum and the contents allowed to drain into the open troughs. Once the herbicide had stopped flowing from the drums, they were allowed to drain for a five minute period after which the interior of each drum was rinsed twice with a total of two gal of diesel fuel. The diesel fuel rinsing drained into the open troughs, combining with the herbicide. After the 12 drums in each set had drained for a minimum of two minutes they were transported to a nearby drum crusher which consisted of a large weight suspended between two vertical I-beams. One drum at a time was crushed along its longitudinal axis and when approximately 30 drums had been crushed they were removed, banded, and stacked together near the crusher.

The liquid herbicide and diesel fuel rinsing from the drums flowed into the two open troughs to a below-grade sump. The material was pumped from this sump into modified fuel tankers that transported 3,000 gal lots to dockside where the material was pumped aboard the M/T Vulcanus.

A total of 24,795 drums of herbicide was processed in this fashion between 27 July 1977 and 23 August 1977. Two 10-hour shifts of approximately 50 men each were used. The workers were civilian employees of a contractor engaged to perform the dedrumming operations. USAF officers monitored all operations. As at NCBC, all workers were provided daily changes of freshly laundered work clothes, and men working within the dedrum facility were provided protective clothing consisting of cartridge respirators, face shields, rubber aprons, rubber gloves and boots. Unlike at NCBC, men on each crew remained in the same job through the dedrumming/transfer operations. A requirement of employment was pre- and post-operational physical examinations similar to those given the workers at the NCBC.

IV. LAND-BASED OPERATIONS MONITORING PROGRAMS

Detailed plans for environmental and occupational monitoring at both sites are contained in Annexes 4 and 5, *Air Force Logistics Command Programming Plan 75-19 for the Disposal of Orange Herbicide* (2). This section outlines only the industrial hygiene and ambient air monitoring programs conducted at each site. These aspects of the environmental and occupational monitoring at each site were very similar. Essentially, the same equipment, methods and procedures were used at both sites. The only significant difference between the two operations was that all sampling at the NCBC site was accomplished by members of the US Air Force Occupational and Environmental Health Laboratory (USAF OEHL), Brooks AFB, Texas, while all sampling at the Johnston Island site was conducted by Battelle Columbus Laboratories (BCL), Columbus, Ohio, under contract to the USAF. An environmental engineer from the USAF OEHL served as Project Officer and monitor of the BCL contract. In general, the industrial hygiene sampling program consisted of daily air samples within the dedrum facilities with rapid analysis (approximately 24-hour turn around time) for 2,4-D and 2,4,5-T. Samples collected for analysis of TCDD were analyzed after-the-fact. The ambient air sampling at various locations and distances from the dedrum facilities included samples for 2,4-D, 2,4,5-T and TCDD analyses, as well as biomonitoring using rapidly growing tomato plants as indicator organisms. Pre-operational and post-operational background sampling was also accomplished.

A. Monitoring Equipment and Procedures

Two different methods were employed for industrial hygiene and ambient air sampling for 2,4-D, 2,4,5-T and TCDD. These procedures have been developed and field tested by the USAF OEHL.

1. 2,4-D and 2,4,5-T

Sampling for 2,4-D and 2,4,5-T was accomplished utilizing Chromosorb(R) 102 as an adsorption medium, a granular polymer well suited for collection of chlorinated hydrocarbon vapors in air (10,11). The polymer was packed in micro-pipet tubes which were then wrapped in new aluminum foil and stored in rubber stoppered test tubes. The sampling apparatus consisted of a Mine Safety Appliance Model G Personnel Sampling Pump. The Chromosorb(R) 102 tubes were connected to the pumps with Tygon(R) or latex rubber tubing. A flow rate of 0.50 liters/minute (l/min) for periods ranging from five to ten hours was used, yielding an air sample volume of approximately 150 to 300 liters. This sampling time corresponded to the length of approximately one-half shift and was expected to yield sufficient adsorption efficiency to permit easy analysis. Flow rates were checked hourly with a calibrated rotameter to insure that 0.50 l/min flow rate was maintained. Where possible the pumps were maintained on constant "high" recharge by providing connections to available 110-volt power supply. When the Chromosorb(R) 102 tubes were removed from the field for lab analysis the individual tubes were wrapped in aluminum foil and returned to their respective rubber stoppered test tubes.

2. TCDD

Air sampling for TCDD was accomplished using benzene as a collection medium. The sampling apparatus consisted of a train of four Greenberg-Smith impingers. The first two impingers were fritted and each contained approximately 350 ml of benzene. The third and fourth impingers were modified by removal of the fritts and contained activated carbon to adsorb vaporized benzene. The two benzene impingers were wrapped with aluminum foil providing a light barrier that would prevent any photo-decomposition of the TCDD collected in the sample. Following the four impingers, an in-line paper filter was attached with Tygon(R) tubing to prevent carbon particles from entering the Millipore pump. The pumps were operated directly from 110-volt AC power and the flow rate was one l/min. The duration of sampling ranged from three to five hours, yielding an air sample volume from 180 to 300 liters. Flow rates were checked hourly using a calibrated rotameter and total volume of air sampled was calculated from these hourly flow rates. The maximum running time of five hours was dictated by ambient temperatures ranging from 71 to 92 degrees F and the saturation limitations of the carbon to adsorb the benzene vapors. Samples were removed from the sampling sites with impinger trains intact in special wooden holders. The benzene was drained into new brown glass jars in a "clean" laboratory area. The impinger glassware was rinsed with benzene into the sample container to collect any materials adhering to the impinger walls. All impinger glassware was rinsed three times with acetone and once with benzene prior to reuse in the field.

3. Biomonitoring

Immature, rapidly-growing, potted tomato plants, *Lycopersicon esculentum*, ranging in size from 6 inches to 18 inches were used as indicator organisms for detecting the presence of Herbicide Orange vapors in air at various locations around the land-based dedrumming, transfer and loading operations. Young tomato plants are known to be very sensitive to phenoxy herbicide vapors (9). The symptoms typical of exposure to Herbicide Orange vapors, known as epinastic growth, is described as a curling and/or twisting of the apical portions of the plants. Depending on level of exposure these symptoms would appear within 24-hours after exposure. Normal procedures included observations, at least once daily, of the tomato plants to record the presence of the epinastic growth symptoms and to water the plants. Relative rating scales were used to describe the levels of damage noted. It was not possible to quantitate the levels of vapor exposure, but the extent to which low parts-per-trillion (ppt) herbicide vapor levels were carried by prevailing winds could be determined.

B. Analytical Procedures and Methodologies

The analytical procedures and methodologies used throughout Project PACER HO were developed, refined, tested and repeatedly used throughout the variety of field exercises conducted by the USAF OEHL over the five year period from 1972 to 1977.

1. 2,4-D and 2,4,5-T

Analysis of Chromosorb$^{(R)}$ 102 air samples was provided by two different laboratories. In the case of the NCBC, samples were analyzed by the U.S. Department of Agriculture Laboratory, Gulfport MS under an interservice agreement. All Johnston Island air samples for 2,4-D and 2,4,5-T were analyzed by the staff of the Battelle Columbus Laboratory team. The methods for analyses of herbicide will be reported elsewhere (4,5).

2. TCDD

The Brehm Laboratory, Department of Chemistry, Wright-State University, Dayton Ohio, analyzed all benzene impinger samples for TCDD as well as many other types of samples and substrates in support of Project PACER HO. The Brehm Laboratory has been under contract with the USAF for several years and has developed unique analytical capabilities in trace analysis for TCDD in a variety of substrates. The analytical methods employed for this project by the Brehm Laboratory have recently been published (8).

V. LAND-BASED MONITORING RESULTS

Detailed results of environmental and occupational monitoring at both sites will be reported elsewhere (4,5). This section outlines only the industrial hygiene and ambient air monitoring results for each site. Suffice to say that all other available data have indicated that there were no adverse environmental impacts on air, water or land resources at either site as a result of land-based dedrumming, transfer operations.

A. NCBC, Gulfport MS

The results of the industrial hygiene and ambient air monitoring programs at the NCBC are summarized below:

1. Industrial Hygiene

The industrial hygiene air sampling results for 2,4-D, 2,4,5-T and TCDD are presented in Table 1. Five operational industrial hygiene samples were collected during each shift from the four corners within the enclosed dedrum facility. Four of these samples were for 2,4-D, 2,4,5-T using Chromosorb(R) 102, while the fifth sample was a benzene impinger in one corner of the facility collected for TCDD analysis. The Chromosorb(R) 102 and benzene impinger samplers were placed in low traffic areas near the four corners of the enclosed facility to prevent interference with work activity within the facility. As shown in Table 1, vapor concentrations of the n-butyl esters of 2,4-D and 2,4,5-T ranged from 7.76 - 141.15 $\mu g/m^3$, respectively. The uniformity of concentrations of herbicide vapors within the dedrum facility is demonstrated by the lack of significant variability of 2,4-D/2,4,5-T data among the four sampling locations. All noted levels were well below the time weighted average Threshold Limit Value (TLV) of 10,000 $\mu g/m^3$ for either 2,4-D or 2,4,5-T as adopted by the American Conference of Governmental Industrial Hygienists (ACGIH). No TCDD was detected in any of the 27 benzene impinger samples. The minimum detectable concentrations for TCDD ranged from 22.4 to 35.9 ng/m^3.

2. Ambient Air

Ambient air samples for 2,4-D/2,4,5-T and TCDD analysis were collected from three different locations. In addition, 29 groups of four tomato plants each were positioned around the dedrumming/transfer operations. The results of these monitoring efforts are presented below:

a. 2,4-D, 2,4,5-T and TCDD. Sampling stations at two locations on the NCBC were established, one at the base fire station approximately 900 feet SW of the dedrum facility and one at the PACER HO Operation Center approximately 1,500 feet E of the dedrum facility. A

TABLE 1. Results of industrial hygiene air samples collected inside the dedrumming facility Project PACER HO NCBC, Gulfport, MS, 24 May - 10 June 1977.

	Sample Location Dedrum Facility Naval Construction Battalion Center (NCBC)			
Parameter	SE Corner	NE Corner	SW Corner	NW Corner
No. of Samples	28	28	14	14
NBE[a]2,4-D ($\mu g/m^3$)				
Range	8.7-141.15	7.86-136.35	7.76-134.9	15.18-105.11
Std Dev	31.45	34.55	36.25	27.01
Mean	52.99	53.72	54.58	51.5
NBE[a]2,4,5-T ($\mu g/m^3$)				
Range	5.52-65.11	5.70-76.36	3.01-79.62	7.59-51.31
Std Dev	14.98	18.57	21.02	12.79
Mean	26.40	29.93	32.39	25.93
TCDD				
No. of Samples	27	0	0	0
Mean	ND[b]	-	-	-

[a] NBE is normal-butyl ester.

[b] ND is non-detectable at minimum detectable concentrations that ranged from <22.4 to <35.9 ng/m^3.

NOTE: The time-weighted Threshold Limit Value for either 2,4-D or 2,4,5-T is 10,000 $\mu g/m^3$. (See text)

third location on the wharf approximately 300 feet north of the ship loading point was also sampled. The results of analyses of these samples are presented in Table 2. As expected, the levels of 2,4-D, 2,4,5-T were significantly (45 to 150 times) lower than were found within the dedrum facility. Filtering of exhaust air from the facility, downwind diffusion/dispersion of released vapors, and the lack of any significant spillage of herbicide outside the facility no doubt accounted for these significantly lower levels. No TCDD was detected at any of the three ambient air sampling stations with the minimum detectable concentrations ranging from approximately 22 to 55 ng/m^3.

b. Biomonitoring. Tomato plants were placed in groups of four in two concentric rings around the dedrum facility at 500 feet and 1000 feet distances. Moderate to severe plant damage was noted along the axis of prevailing winds in the inner ring (500 feet). Slight to moderate plant damage was noted in the corresponding outer (1000 feet) ring. In addition, several sets of four plants were set up along the NCBC perimeter fence. In two cases test plants along the base perimeter showed only minimal damage. One set of plants was also placed on the dock 300 feet inland from the loading operations. No damage was noted at this location.

B. Johnston Island

The results of the industrial hygiene and ambient air monitoring programs at Johnston Island are summarized below. There were two distinct loading operations during the Johnston Island phase of the project. The first dedrum/transfer (first loading) operation was conducted from 27 July 1977 to 5 August 1977, and the second loading from 17 August 1977 to 23 August 1977.

1. Industrial Hygiene

The industrial hygiene sampling of the Johnston Island operations differed from the sampling at the NCBC. The facility was larger and open to natural ventilation and the dedrum operations were far different as described earlier. Because of these and other factors the industrial hygiene sampling program was modified to include true "breathing zone" samples for 2,4-D, 2,4,5-T from selected worker positions. In general, there were three worker positions evaluated using the Chromosorb(R) 102 tubes. These positions were selected after an analysis of all positions revealed that these worker locations represented the greatest possibility of receiving a significant exposure. The first was the position occupied by those workers who punched the vent holes in each drum. When the vent holes were punched internal pressure in many drums was released, and there was a possibility of elevated exposures to workers in these positions. The second worker position evaluated was that occupied by the workers who punched the several drain holes in each drum, and the third position was the operator of the sump pump. In the latter two cases, these workers were close to open troughs of flowing Herbicide Orange. In addition to these "breathing zone" samples, air samples within the dedrum facility were also collected for 2,4-D, 2,4,5-T and TCDD. Tables 3, 4 and 5 present the results of these sampling programs.

TABLE 2. Results of ambient air samples collected at Gulfport MS, Project PACER HO, 24 May - 10 June 1977.

	Sample Location NCBC, Gulfport, MS		
Parameter	Fire Station	Ops Center	Wharf
No. of Samples	29	30	28
NBE[a] 2,4-D ($\mu g/m^3$)			
Range	0.09-5.76	0.13-3.88	0.07-2.41
Std Dev	1.20	1.00	0.53
Mean	1.17	1.09	0.52
NBE[a] 2,4,5-T ($\mu g/m^3$)			
Range	0.04-3.36	0.34-1.97	0.01-1.45
Std Dev	0.85	0.49	0.32
Mean	0.52	0.34	0.21
TCDD			
No. of Samples	27	27	23
Mean	ND[b]	ND[b]	ND[b]

[a] NBE is normal butyl ester.

[b] ND is non-detectable at minimum detectable concentrations that ranged from <21.9 to 55.2 ng/m^3.

NOTE: The time-weighted Threshold Limit Value for either 2,4-D or 2,4,5-T is 10,000 $\mu g/m^3$. (See text)

TABLE 3. Results of industrial hygiene air samples collected inside the dedrumming facility, Project PACER HO Johnston Island, first loading 27 July - 5 August 1977.

Parameter	Sample Location Dedrum Facility Johnston Island, First Loading		
	SW Corner	NW Corner	E Wall
No. of Samples	3	3	3
NBE[a] 2,4-D ($\mu g/m^3$)			
Range	12.8-16.0	4.79-18.33	0.50-2.58
Std Dev	1.77	7.30	1.37
Mean	14.84	9.99	1.03
NBE[a] 2,4,5-T ($\mu g/m^3$)			
Range	6.92-8.84	2.26-8.28	-
Std Dev	1.05	3.24	-
Mean	8.12	4.58	-
TCDD			
No. of Samples	4	0	0
Mean	ND[b]	-	-

[a] NBE is normal butyl ester.

[b] ND is non-detectable at minimum detectable concentrations that ranged from <8.06 to <13.89 ng/m^3.

NOTE: The time-weighted Threshold Limit Value for either 2,4-D or 2,4,5-T is 10,000 $\mu g/m^3$. (See text)

TABLE 4. Results of industrial hygiene air samples collected inside the dedrumming facility Project PACER HO, Johnston Island, second loading, 17 - 23 August 1977.

	Sample Location Dedrum Facility Johnston Island, Second Loading	
Parameter	[illegible] Corner	NW Corner
No. of Samples	1	1
NBE[a] 2,4-D ($\mu g/m^3$)	18.78	6.60
NBE[a] 2,4,5-T ($\mu g/m^3$)	7.35	2.27
TCDD		
No. of Samples	5	0
Mean	ND[b]	-

[a] NBE is normal butyl ester.

[b] ND is non-detectable at minimum detectable concentrations that ranged from <6.64 to <23.41 ng/m^3.

TABLE 5. Results of industrial hygiene "breathing zone" samples collected inside the dedrumming facility Project PACER HO, Johnston Island.

	Sample locations Dedrum Facility Johnston Island, (See Text)		
Parameter	Vent Punchers	Drain Punchers	Pump Operator
	First Loading (27 July - 5 August 1977)		
No. of Samples	8	10	5
NBE[a] 2,4-D ($\mu g/m^3$)			
Range	2.14-30.8	7.64-19.18	6.11-26.78
Std Dev	8.35	5.73	8.18
Mean	17.92	19.18	14.36
NBE[a] 2,4,5-T ($\mu g/m^3$)			
Range	0.57-16.1	3.79-13.6	2.43-11.48
Std Dev	4.52	2.95	3.61
Mean	8.70	9.54	6.32
	Second Loading (17 - 23 August 1977)		
No. of Samples	12	7	0
NBE[a] 2,4-D ($\mu g/m^3$)			
Range	8.38-40.28	15.96-38.0	-
Std Dev	10.47	8.53	-
Mean	23.20	23.04	-
NBE[a] 2,4,5-T ($\mu g/m^3$)			
Range	6.49-22.22	8.82-22.53	-
Std Dev	6.06	5.20	-
Mean	13.21	13.68	-

[a] NBE is normal butyl ester.

NOTE: The time-weighted Threshold Limit Value for either 2,4-D or 2,4,5-T is 10,000 $\mu g/m^3$. (See text)

The levels noted within the dedrum facility at Johnston Island were on the order of two to five times lower than those noted at the NCBC, Gulfport MS. These lower concentrations probably resulted from much greater dilution by natural ventilation of the open facility at Johnston Island. Needless to say, the noted levels of 2,4-D and 2,4,5-T were well below the ACGIH TLV of 10,000 μg/m^3. No TCDD was detected in any of the samples analyzed.

2. Ambient Air

Ambient air samples for 2,4-D/2,4,5-T and TCDD analyses were collected from three different locations. In additon, 14 groups of four tomato plants were positioned at selected locations around the dedrum/transfer operations. The results of these monitoring efforts follow.

a. 2,4-D/2,4,5-T and TCDD. One downwind and two upwind sampling stations were established. The downwind site was located approximately 300 feet west of the dedrum facility. The two upwind sites were the fire station approximately 4,000 feet SE and the weather station approximately 6,000 feet ESE of the dedrum facility. The results of downwind and upwind ambient air sampling sites are presented in Tables 6 and 7, respectively. As was expected, the levels of 2,4-D/2,4,5-T noted at the downwind site were somewhat lower than those levels noted within the dedrum facility. The relatively higher levels noted for the second loading as compared to the first loading are not explainable. These levels, however, are well below the TLV. No TCDD was detected in any of these samples.

b. Biomonitoring. Tomato plants were placed at 14 biomonitoring stations on Johnston Island. Four of these sites were downwind of the dedrumming facility and the remaining ten locations were all upwind. Throughout the two periods of dedrumming operations all the downwind sites displayed slight to severe herbicide induced damage. There was only slight damage noted on two days at one of the upwind sites. The results of the tomato plant bioassay indicate that during the dedrumming operations concentrations of Herbicide Orange did not occur upwind of the dedrumming facility at sufficient concentrations to affect the tomato plants.

VI. SUMMARY AND CONCLUSIONS

As part of the environmental and occupational monitoring programs, the USAF accomplished industrial hygiene and ambient air sampling of all land-based dedrumming/transfer operations of Project PACER HO, the USAF project to dispose of 2.22 million gal of Herbicide Orange.

The results of these sampling programs revealed that under the worst case noted, the levels of 2,4-D and 2,4,5-T vapors were well below the TLV for each of these materials. The noted levels were at least two and in most cases three orders of magnitude below the TLVs. TCDD was not detected in any air samples.

TABLE 6. Results of downwind ambient air samples collected at Johnston Island, Project PACER HO, 27 July - 23 August 1977.

	Downwind Ambient Air Sampling	
Parameter	First Loading (27 Jul-5 Aug 77)	Second Loading (17-23 Aug 77)
No. of Samples	14	11
NBE[a] 2,4-D ($\mu g/m^3$)		
Range	1.92-25.5	5.79-32.67
Std Dev	5.99	7.73
Mean	6.21	12.51
NBE[a] 2,3,5-T ($\mu g/m^3$)		
Range	0.82-17.88	1.89-14.0
Std Dev	4.33	3.46
Mean	3.27	5.12
TCDD		
No. of Samples	2	2
Mean	ND[b]	ND[b]

[a] NBE is normal butyl ester.

[b] ND is non-detectable at minimum detectable concentrations that ranged from <11.68 to <21.0 ng/m^3.

NOTE: The time-weighted Threshold Limit Value for either 2,4-D or 2,4,5-T is 10,000 $\mu g/m^3$. (See text)

TABLE 7. Results of upwind ambient air samples collected at Johnston Island, Project PACER HO, 27 July - 23 August 1977.

	Weather Station		Wharf Station	
	First Loading[b]	Second Loading[c]	First Loading[b]	Second Loading[c]
No. of Samples	11	11	11	7
NBE[a] 2,4-D ($\mu g/m^3$)				
Range	Trace-0.67	ND-2.54	Trace-1.09	-
Std Dev	0.39	0.77	0.42	0
Mean	0.25	0.23	0.29	0
NBE[a] 2,4,5-T ($\mu g/m^3$)				
Range	-	Trace	-	-
Std Dev	0	0.34	0	0
Mean	0	0.10	0	0
TCDD				
No. of Samples	0	1	1	0
Mean	-	ND[d]	ND[e]	-

[a] NBE is normal butyl ester.

[b] First Loading 27 July - 5 August 1977.

[c] Second Loading 17-23 August 1977.

[d] ND is non-detectable at the minimum detectable concentration of <8.52 ng/m^3.

[e] ND is non-detectable at the minimum detectable concentration of <20.34 ng/m^3.

NOTE: The time-weighted Threshold Limit Value for either 2,4-D or 2,4,5-T is 10,000 $\mu g/m^3$. (See text)

Biomonitoring using tomato plants revealed that low-level vapors of Herbicide Orange were dispersed and diffused downwind of the land-based dedrumming/transfer operations at both sites. No adverse environmental impact resulted from these operations.

Approximately 200 personnel carried out the dedrumming activities at the NCBC, Gulfport MS and at Johnston Island. Comparisons of available pre- and post-operational medical examinations of military personnel involved have revealed no apparent physical effects as a result of these activities.

CHAPTER II
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CHAPTER III
ENVIRONMENTAL FATE OF 2,4-D, 2,4,5-T AND TCDD

I. INTRODUCTION

Chapter I was devoted to the topics of types and quantities of herbicides sprayed in South Vietnam and their handling and application. Emphasis was placed on those factors that may have influenced human exposure to the herbicides prior to actual spray applications.

This chapter will focus primarily on the fate of the phenoxy herbicides sprayed in South Vietnam and on the contaminant TCDD. This is appropriate since 94 percent of all herbicides disseminated in South Vietnam were phenoxy herbicides (53 percent 2,4-D and 41 percent 2,4,5-T). The extreme toxicity of the contaminant, and its associated biological effects, require that all available data be reviewed in an attempt to determine the potential adverse human effects this compound may have had on the population at risk in South Vietnam. What happens to the individual compounds physically, chemically and biologically in the environment will significantly influence the route of exposure, the duration of exposure and the total dose (or level) of that exposure to the population at risk. Again, as noted in Chapter I, the population at risk will be confined to personnel of the U.S. military forces.

The expression of units of weight, area, or volume has not been standardized between various publications cited in this Chapter.

II. THE ENVIRONMENTAL FATE OF THE PHENOXY HERBICIDES

A. Physical/Chemical Factors Influencing Disappearance of Herbicides

1. Fate in Air

Harrigan (27) reported that in a test program evaluating the dissemination characteristics of the A/A 45 Y-1 Spray System, the mean recovery of Herbicide Orange by ground sampling methods from six missions flown under operational parameters typically used in South Vietnam was 87 percent. The remaining 13 percent may have been undetected due to sampling technique or may have failed to impact the sampling array due to drift or volatility. The mean particle size for the six missions flown was 367 micron (μ). Harrigan (27) in the above test program with Herbicide Orange, found the following droplet size distribution in the mean percent mass recovered:

Particles less than 100μ	1.9 percent
Particles 100 to 500μ	76.2 percent
Particles greater than 500μ	21.9 percent

The recovery of 87 percent of the Herbicide Orange disseminated is in agreement with Plimmer (50) who reported that deposition of 80 percent of particles greater than 200μ in size takes place in short downwind distances, whereas those of diameter less than 5μ may drift for miles.

The aerial application of Herbicide Orange also presented an opportunity for volatilization since spray drops evaporate during their fall. This was recognized by Grover et al (25), who examined the relative potential for drift of volatile and nonvolatile formulations of 2,4-D under conditions of typical agricultural application. The ground application system employed by Grover et al resulted in only 2.8 percent of the total spray having a particle size less than 200μ. The mass of the formulation drifing as droplets was similar (3 to 4 percent) for either the volatile (n-butyl ester) or nonvolatile (dimethylamine) formulation of 2,4-D. However, for the butyl ester, in addition to droplet drift, within the first 30 minutes after spraying 25 to 30 percent of the material was collected as vapor drift in air samplers up to 246 feet (ft) downwind from the point of application.

The data by Grover et al (25) may suggest that although high percentages of Orange particles were intercepted by the vegetation, a significant amount of the material may have rapidly volatilized and moved in the air within the jungle canopy. This is in accord with what Brown (12) had first proposed in 1962 when he recommended the use of the esters of 2,4-D and 2,4,5-T for defoliation in South Vietnam.

> Better effect can be achieved on a susceptible tree if all its leaves receive a few drops of chemical as opposed to only one side or only the very top of the tree receiving all the chemical. In this connection, forms of the chemical known as volatile esters were requested subsequently in order to achieve more uniform coverage within a forest canopy.

2. Fate on Vegetation

Approximately 85 percent of all the 2,4-D and 2,4,5-T sprayed in South Vietnam was with the C-123/A/A 45 Y-1 Spray System [estimate based on data by Irish et al (31), National Academy of Science (15), Craig (16), and Chapter I.] Klein and Harrigan (36) found that in five standard Orange missions the statistical mean value for maximum swath width having a deposition rate that would result in acceptable defoliation was 260± 20 ft. Thus, a typical 1,000 gallon (gal) sortie in South Vietnam would have effectively defoliated an area of approximately 346 acres (A). Data by Tschirley

(58) suggested that a multicanopy forest would intercept at least 94 percent of all the spray droplets. It is therefore reasonable to assume that if the entire 1,000 gal of Orange fell within the 346 A area, 940 gal of Orange would have been deposited on the canopy vegetation, and 60 gal deposited at ground-level on the soil or small herbaceous understory. The actual ground-level deposition may then have been 0.17 gal/A or 1.4 pounds (lb) of 2,4-D/2,4,5-T per acre (60 gal/346 A = 0.17 gal/A x 8.14 lb active ingredient/gal = 1.4 lb 2,4-D/2,4,5-T per A). In the United States, mixtures of these phenoxy herbicides are routinely applied at 2 lb/A. If time after application was the same, then military personnel moving through defoliated forests in South Vietnam probably would have encountered the same amount of herbicide as would a rancher in the United States walking through defoliated brush-infested ranch land.

Once the herbicide is intercepted by the vegetation, numerous physical and chemical barriers influence the amount of herbicide that is absorbed, transported and accumulated. In Volume 2 (Weed Control) of a special series on the principles of plant and animal pest control, the National Academy of Science (49) reviewed the physical and chemical barriers which intervene between application of a herbicide and its ultimate effect on the plant. They found that, in general, both the upper and lower leaf surfaces absorb herbicides. Usually, the lower epidermis is penetrated more readily, but not all areas of either surface are equally permeable. The penetration of the phenoxy herbicides into most foilage is by diffusion through the cuticle (cuticular entry). Warm temperatures that are not excessive and high humidity may actually promote the entry. Because the cuticle and the cell walls upon which the cuticle is deposited contain chemically nonpolar materials that are slightly electronegative, nonpolar herbicides (e.g., Orange and Purple) tend to be absorbed into leaves faster than polar herbicides. Cuticular penetration by the esters of 2,4-D or 2,4,5-T may occur within 30 minutes of their application.

3. Fate in Soils

Hamaker (26) has reviewed the physical and chemical factors that influence fate of herbicides in soil. These include soil adsorption, hydrodynamic dispersion and diffusion, adsorption dynamics and evapotranspiration. The phenoxy herbicides, for example, have low adsorption coefficients and thus tend to leach in a soil profile. The actual amount of leaching, however, will vary from soil to soil, mainly in response to the organic carbon content. Moreover, only the herbicide free in the soil water will be carried down by descending water.

Crosby (18), in reviewing nonbiological degradation of the phenoxy herbicides in soil, reported that the isopropyl, butyl and isooctyl esters of 2,4-D had a half-life of about 100 hours (h) in neutral soil water (although hydrolysis was almost instantaneous in the presence of a base or a suspension of any of several soils at pH 7.0-7.5). Moreover, many of the phenoxy herbicides, e.g., 2,4-D, will readily undergo oxidation, reduction and substitution (notably hydrolysis) in aqueous solutions when activated by sunlight in air. The end product of the photodegradation of 2,4-D is humic acid (17). Although 2,4,5-T absorbs some ultraviolet light in sunlight, the amount is small and this herbicide is relatively unreactive (only 7 percent was hydrolyzed in 48 h). However, the presence of ferric salts or zinc oxides in the soil water will result in an increase in photolysis rate (17).

Another nonbiological factor that determines the soil persistence of the phenoxy herbicides is their tendency to volatilize from the soil complex. Plimmer (50) noted that some volatilization will occur whether or not water is evaporating from the soil. However, a reduction in soil moisture content will decrease the pH of soil. This will favor the undissociated form of 2,4-D and 2,4,5-T and their potential for vapor loss may be increased.

B. Biological Degradation of the Phenoxy Herbicides

1. Fate in Plants

Loos (40) has recently reviewed the degradation of phenoxy herbicides in plants.

In general, because of the widely different degradative pathways, the phenoxy herbicides do not persist in plants. However, Muzik (44) has reported that in some plants, for example, tomato, unmetabolized 2,4-D may be bound to cellular membranes and persist for two or three months.

2. Fate in Soils

There is considerable evidence available to show that the phenoxy herbicides are rapidly decomposed in soils (5). Goring et al (23) in reviewing principles of pesticide degradation in soil noted that 2,4-D may undergo at least 6 different types of oxidation reactions, 1 reductive reaction, 1 hydrolytic reaction and 4 conjugative reactions. Because of this ability to readily undergo transformation, 2,4-D has been classed as a non-persistent pesticide since the estimated time required for 50 percent to disappear from soil was <0.5 months. However, 2,4,5-T has been classed as a slightly persistent pesticide since the time required for 50 percent disappearance was 0.5 to 1.5 months.

If 2,4-D were applied to a moist loam soil under summertime temperature at a rate of 0.5 to 3 pounds/acre (lb/A), it would disappear in 7 to 30 days (37). If applied at rates of 4 to 55 lb/A, it would probably disappear in one to three months (22). If 2,4-D were applied to the soil at a concentration of 500 ppm and disappeared at a rate proportional to the breakdown of 55 lb/A, the calculated time would be 5.6 years. However, there is evidence that a more realistic time for inactivation of 500 ppm would be less (4).

Persistence of 2,4,5-T in soils is usually two to three times longer than 2,4-D (22), and very few organisms have been identified as having the ability to breakdown the 2,4,5-T molecule (2). Newton (46) has calculated from studies on the kinetics of degradation by microorganisms that 2,4,5-T has a half-life of seven weeks in the forest floor. Investigations by Winston and Ritty (59) and Reigner et al (51) indicated that both 2,4-D and 2,4,5-T are decomposed to form carbon dioxide, inorganic chlorides and water; objectionable chlorophenols are not end-products of this decomposition. Further supporting evidence has been provided by Reinhart (52). The upper half of a 60 acre timber watershed in northern West Virginia was logged and treated with 2,4,5-T ester to kill all vegetation. The volume of herbicide that was applied was 1,325 gal on 30 acres (418 liters/ha). Almost 790 gal of this were potential contaminating materials: about 740 gal of diesel oil and 50 gal of a commercial formulation of 2,4,5-T (313 pounds acid equivalent). Reinhart found no odor contaminants (phenols or catechols) in the numerous water samples taken from the stream draining the treated watershed.

In relation to the effects of herbicides on the soils of South Vietnam, the National Academy of Science published a report by Blackman et al (11) on persistence and disappearance of herbicides in tropical soils. The 1974 report stated a number of general conclusions, namely:

1. The behavior of herbicides in the soils of South Vietnam was similar to that reported for soils elsewhere.

2. Only where 2,4-D and 2,4,5-T were applied in very massive doses; e.g., at the Pran Buri Calibration Grid in Thailand at rates in the magnitude of 1,000 lb/A, were there still residues (10 years following application) in concentrations above the threshold likely to induce phytotoxic symptoms in some plant species.

3. When applied to mangrove soils at total doses approaching 10 lb/A of 2,4-D and of 2,4,5-T, the level of herbicide residue at the end of 30 weeks had no effect on the establishment of two major mangrove species.

4. In geographical areas subjected to one or two military herbicide missions 1.5 years before sampling, no soil phytotoxic residues could be detected.

5. Soils that received a directed application of Herbicide Orange at the rate of 27 lb/A safely supported the growth of crops sensitive to 2,4-D or 2,4,5-T four to six months following application.

6. Claims that the herbicides rendered the soil sterile were without any foundation.

Byast and Hance (14) have studied the degradation of 2,4,5-T by South Vietnamese soils incubated in the laboratory. Although care must be exercised in extrapolating laboratory results to field situations, their results suggested that the four Vietnamese soils studied were inherently capable of degrading 2,4,5-T at levels roughly twice the rate of military application in Vietnam.

In support of feasibility tests for the soil disposal of surplus Herbicide Orange, the Air Force established a field study in 1972 on the Air Force Logistics Command Test Range, Hill Air Force Base, Utah. The study consisted of replicated plots subsurface injected with concentrations of either 1,000, 2,000, or 4,000 lb herbicide/A. Soil samples were taken by Stark et al (56) three times throughout 1973, and microbial species present (bacteria, actinomycetes and fungi) were determined. Bacterial counts were higher for soils with greater concentrations of the herbicide and with greater moisture content; i.e., those samples collected in midwinter from the 4,000 lb/A plots. Herbicide Orange, in any concentration, had no significant effect on mycoflora. Arnold et al (4) monitored the herbicide levels in these plots. They sampled the plots on eight occasions from 1973 through 1975 and determined the concentrations of the n-butyl esters and free acids of both 2,4-D and 2,4,5-T. They suggested that at such massive application rates (soil concentrations greater than 10,000 ppm) and in an alkaline desert environment, the half-life of 2,4-D and 2,4,5-T appeared to be in the range of 150 to 210 days.

The cooperative studies by Stark et al (56) and Arnold et al (4) have shown that the application of 2,4-D and 2,4,5-T at massive rates not only did not sterilize the soil, but indeed stimulated the growth of certain microflora, and this stimulation may have contributed to the degradation of the herbicide.

C. Accumulation and Metabolism of Phenoxy Herbicides in Animals

A detailed review of the toxicity, distribution and fate of 2,4-D and 2,4,5-T in animals is provided in Chapter IV. Some general observations on the metabolism of the phenoxy herbicides have recently been published by Leng (39). She reported that residues of the phenoxy herbicides in treated food or feed crops were readily absorbed in the gut of animals and were excreted rapidly in the urine, largely as unchanged phenoxy acid. Some conjugation occurred, particularly at higher dosage levels, but the basic structure of the herbicide was not readily altered in animals. The ether linkage can be cleaved by bacterial action in the rumen but the rate of cleavage depended on the chemical structure of the phenoxy compound. The rate of clearance of residues from the body was dependent upon dosage level, particularly if the renal threshold was exceeded. Leng (39) concluded that residue levels were considerably lower in muscle, milk, and cream than in liver and kidney, but that all residue levels rapidly declined after withdrawal of animals from treated feed. Residues of phenol metabolites were present in milk, liver and kidney of animals fed high doses of 2,4-D and 2,4,5-T.

III. THE ENVIRONMENTAL FATE OF TCDD

A. Analytical Limitations

Statements on the fate of TCDD in the environment are predicated upon the detection in environmental substrates. Prior to 1973, the detection limit for TCDD, was 0.1 ppb for soils and 0.05 ppm for biological tissue (60). As noted by Kearney et al (35) and Dost et al (23), a 1 lb/A application of 2,4,5-T containing 0.1 ppm TCDD applied directly to the soil could result in a maximum of 0.1 parts per trillion (ppt) in the top 15 cm of soil. Likewise, Baughman and Meselson (9) have calculated that environmental monitoring of food chains for buildup of TCDD would require a level of detection of 1 ppt. For a 1 gram sample of biological tissue, this would require a limit of detection of 1 picogram (pg) (10^{-12} gram). Highly sophisticated instrumentation is required to obtain these low detection limits. However, another one of the limiting factors, even with appropriate instrumentation, has been the need for cleanup techniques applicable to a wide variety of environmental samples.

Recently (1977), Hummel (30) has reported on a technique suitable for permitting the detection of ppt residue levels of TCDD. Using this technique, Hummel has analyzed a wide array of environmental substrates. These have included analyses of whole fish, fish muscle, rat and mouse liver, mouse pelts, bird liver and stomach, insects, diving beetles, seeds, soil, water, and bovine and human milk.

Largely due to the analytical limitations noted above, the quest for environmental data on TCDD began with laboratory experiments. The use of radiolabeled preparations were invaluable in these studies. There has been considerable interest placed on the analysis of TCDD in field samples; e.g., fish and human milk from South Vietnam (7), bovine fat, liver and milk from the Western United States (3,41), and rice from Arkansas (30, 55).

B. Laboratory Studies of TCDD

Two model ecosystem studies (33, 42) have been conducted in an attempt to simulate the mode of entry of TCDD into water with the subsequent exposure of several organisms representing parts of natural food chains. These systems were not designed to determine the effects of TCDD on the organisms but rather, how does TCDD behave when subjected to likely environmental conditions.

Matsumura and Benezet (42) introduced ^{14}C-TCDD in the form of residues on sand into an aquatic ecosystem containing brine shrimp, mosquito larvae and fish. The results indicated that the rate of pick-up was extremely low in brine shrimp and fish under the experimental conditions. Mosquito larvae, which were bottom feeders, showed a faster rate of TCDD pick-up. They concluded that because of TCDD's low solubility in water and its low partition coefficient in lipids, it was not likely to accumulate in as many biological systems as DDT.

Isensee and Jones (33) exposed several organisms to ^{14}C-TCDD for up to 31 days to determine the distribution and bioaccumulation potential in the aquatic environment. TCDD accumulation by all organisms was directly related to water concentration (0.05-1330 ppt) and ranged from 2.0×10^4 to 2.6×10^4 times the water concentration for snail, mosquito fish and daphnids and averaged 4.9×10^3 for duckweed, algae and catfish. No metabolities of TCDD were found in submerged soil, water, snails, mosquito fish or catfish. Isensee and Jones further noted that most (85-99 percent) of the ^{14}C-TCDD originally added to the ecosystem remained in the soil at the end of the experiment. Total recovery for the ecosystem averaged 92.2 percent, indicating that TCDD was very stable during this study.

From the model ecosystem data, it has been concluded that TCDD is taken up by an organism and retained (bioaccumulation). The accumulation results in TCDD concentrations in the environment (bioconcentration). The food chain studies do not suggest, however, that TCDD is biomagnified; i.e., organisms at successive trophic levels do not exhibit an ascending order of TCDD concentrations in their tissues. Neither of the model ecosystem studies reported toxic effects from the bioconcentration of the TCDD. Both studies, however, were of short duration and the water concentration was generally low, although in one experiment by Isensee and Jones (33) the water concentration exceeded 1 ppb and mosquito fish and catfish accumulated concentrations greater than 1.4 ppm TCDD for 3 and 6 days, respectively.

Miller et al (43) conducted chronic toxicity tests to assess the hazard to aquatic organisms exposed to TCDD in water or food. They evaluated three species of fish: guppies, coho or silver salmon, and the rainbow trout; and three aquatic invertebrates: a snail, a worm and mosquito larvae. Their conclusions were that TCDD in water or food was toxic to fish. The effects of exposure for 24-96 h of young salmon to TCDD in water at levels greater than 23 ng/g (23 ppb) were irreversible, and death resulted in 10-18 days. Duration of exposure was less important than level of exposure except as threshold response level was approached. The critical exposure period was somewhat less than 24 h in static water toxicity tests in which TCDD concentrations changed markedly with time. Small fish were more sensitive than large fish on an equivalent exposure level basis. TCDD in food at 2.3 ppm markedly reduced growth of young rainbow trout (10/aquaria) exposed to 6.3 μg TCDD per tank per week for 4 weeks. TCDD at 0.2 ppb had no effect on pupation of the mosquito larvae, but reduced the reproductive successes of the pulmonate snail and the oligochaete worm.

Norris and Miller (48) have conducted additional bioassay tests with guppies. Exposure of guppies to concentrations of TCDD equal to or greater than 0.1 ppb for 120 h caused complete mortality in approximately 30 days. Duration of survival was significantly and positively correlated with body lengths.

Beatty et al (10) administered larval and adult forms of the American bullfrog doses of TCDD varying from 25 to 1,000 μg/kg. Doses of TCDD as high as 1 mg/kg failed to have any significant effect upon survival or completion of metamorphosis in tadpole. Doses of TCDD up to 500 μg/kg had no effect on survival of adult frogs. Histopathological examination of various tissues from the metamorphosed tadpoles and adult frogs revealed no abnormalities.

In one of the first laboratory studies of TCDD in soil, Helling (28) found that TCDD was immobile when evaluated by soil thin-layer chromatography. In laboratory leaching studies, Matsumura and Benezet (42) found that virtually no TCDD leached from soil columns of sand or sandy loam.

Kearney et al (34) have determined the persistence of TCDD after 20, 40, 80, 160 and 350 days in Hagerstown and Lakeland soils receiving 1, 10 and 100 ppm TCDD. After 1 year, 56 and 63 percent of the originally applied TCDD was recovered in the Hagerstown and Lakeland soils, respectively. Thus, the half-life was estimated to be about 1 year. Furthermore, TCDD could not be detected after 70 days in soils receiving 10, 100 or-1,000 ppm 2,4,5-trichlorophenol, suggesting that TCDD was not biosynthesized by microbial condensation reactions. The long half-life of TCDD suggested to Kearney et al (34) that it was not readily metabolized by soil microorganisms. This observation was in keeping with what Matsumura and Benezet (42) found. They evaluated 100 microbial strains, which had previously shown the ability to degrade persistent pesticides, for their ability to degrade TCDD. Only 5 of 100 organisms showed some ability to degrade this compound, suggesting that microbes capable of degrading TCDD were rather rare in nature. Helling et al (29), in reviewing the previous studies, concluded that persistence of TCDD was not surprising since it is an insoluble, nonpolar, chlorinated molecule, devoid of biologically labile functional groups.

Isensee and Jones (32), in laboratory studies determined the uptake of TCDD from soil by two crop species. Lakeland sandy loam, a soil with low adsorptive capacity, was treated with ^{14}C-TCDD at the rate of 0.10 and 0.06 ppm, respectively. Oats or soybeans were grown in this soil and their tops were harvested at intervals to maturity. All tissue ^{14}C-activity was expressed on the basis of the original compound. Oats and soybeans accumulated in their tissue less than 0.15 percent of the TCDD present in the soil. Isensee and Jones (32) also evaluated the fate ot TCDD when applied to foliage. Uniform quantities of ^{14}C-TCDD were applied to the center leaflet of the first trifoliate leaf of 3-week-old soybean plants. The first leaf blade of 12-day-old oat plants was treated with ^{14}C-TCDD only. Results indicated that TCDD was not translocated beyond the treated leaflet. An average of 94 percent of the TCDD remained on soybean leaves for 21 days, but the amount continuously decreased on oat leaves. Although Isensee and Jones suggested that volatilization was a key factor in the disappearance of TCDD from foliage, Crosby and Wong (19) have suggested that photodegradation of the dioxins was a plausible explanation.

In an uptake study similar to that of Isensee and Jones (32), but using sorghum, Cupello and Young (20) found that the rate of uptake of TCDD from a Ulysses sandly loam soil was approximately one millionth of one percent of the amount of TCDD in the soil.

Nash and Beall (45) have recently (1978) completed a study on the fate of TCDD in the plants, soil, water and air of a microagroecosystem. Tritium-labeled TCDD at concentrations of 44 or 7,500 ppb was applied to a bluegrass turf microagroecosystem using an emulsifiable concentrate form of the isooctyl ester of 2-(2,4,5-trichlorophenoxy) propionic acid (Silvex) as a carrier. They found that:

1. TCDD concentrations in water leached through soil were below the analytical detection limit (10^{-16} g/g water).

2. TCDD concentrations on grass were initially 20 ppt (10^{-12} g/g grass), but after four weeks were at or below 1 ppt. The half-life was approximately six days.

3. TCDD concentrations in or on soil were less than 0.2 ppt and most (80 percent) was near the soil surface (0-2 cm).

4. TCDD concentrations in air were (immediately after application) less than 100 fg/m^3 (femtogram - 10^{-15}g/m^3) and after four weeks decreased to <3 fg/m^3.

5. TCDD, or its degradation products, concentrations in earthworms were less than 0.3 ppt.

6. The major repositories for TCDD were the soil and thatch.

Nash and Beall (45) concluded that volatilization (approximately 10 percent) of TCDD was a major pathway of dissipation from the microagroecosystem chamber. However, once TCDD was volatilized it dechlorinated in the direct sun and apparently even in shade outdoors or when the sun was filtered with glass in the chambers. Thus, TCDD is sensitive to photodechlorination in the vapor phase even without the presence of ultraviolet light.

C. Field Studies of TCDD

1. Residue in Aquatic Ecosystems

Several monitoring studies for TCDD in aquatic organisms have been conducted. Baughman and Meselson (8) reported finding TCDD concentrations of 70 to 810 parts per trillion (ppt) in fish from rivers of interior Vietnam and concentrations of 18-79 ppt in fish and shellfish along the seacoast of South Vietnam. Their samples were collected in 1970 and analyzed 2-1/2 years later by their method and instrumentation. Zitko (65) and Zitko et al (66) did not detect dioxins in a wide assortment of aquatic organisms collected from the St. John River, New Brunswick or the Bay of Fundy, Canada. Their detection limits, however, were between 0.1 and 1.0 μg/g of tissue. Shadoff et al (55) have examined fish (bass and catfish) from a reservoir in a rice-growing region of Arkansas, where 2,4,5-T had been used annually for more than 20 years. Likewise, fish (walleyes and catfish) were obtained from a reservoir in West Texas where 2,4,5-T had been used for brush control over the past 20 years. No TCDD was detected in any of the samples with a minimum range of 10 ppt.

Young et al (62) reported on species diversities and food chain studies conducted in two aquatic ecosystems draining a unique one-square mile military test area (Test Area C-52A, Eglin AFB, Florida) that received 161,000 pounds 2,4,5-T and 170,000 pounds 2,4-D herbicide during the period 1962-1970. Significant levels (10-710 parts per trillion) of TCDD were found in 1973 within the top six inches of the test area soil. Erosion of soil occurred into a pond on the test area and into a stream immediately adjacent to the area. TCDD levels of 10-35 ppt were found in 1974 in silt of the aquatic systems, but only at the point where eroded soil entered the water. Species diversity studies of the stream were conducted in 1969, 1970, 1973 and 1974. Insect larvae, snails, diving beetles, crayfish, tadpoles and major fish species (by body parts) from both aquatic systems were analyzed for TCDD. Species diversity studies indicated no significant change in the composition of ichthyofauna between these dates or a control stream. Concentrations of TCDD (12 ppt) were found in only two species of fish from the stream, sailfin shiner and mosquito fish. The sample of mosquito fish consisted of bodies with heads and tails removed. Two samples of sailfin shiner were analyzed: one containing viscera only and the other bodies less heads, viscera and caudal fins. Only the viscera contained TCDD. Samples of skin, muscle, gonads, and gut were obtained from spotted sunfish, from the test grid pond. Levels TCDD in those body parts were 4, 4, 18 and 85 ppt, respectively. Gross pathological observations of the sunfish revealed no significant lesions or abnormalities.

2. Residues in Soils

The National Academy of Science (15) reported finding TCDD concentrations of <1.2 to 23.3 parts per billion (ppb) in soil of the Pran Buri Calibration Grid (Thailand), an area used in calibrating RANCH HAND aerial equipment. Woolson et al (60) found no residues in 1971 in Lakeland sand which had received 947 lb/A of 2,4,5-T during 1962-1964. These unusually high doses resulted from testing of aerial application equipment at Eglin AFB, Florida. Although analysis of the applied material was not conducted, 2,4,5-T made prior to 1968 probably contained enough TCDD to be detected throughout the 1-yard of soil profile sampled. Woolson et al suggested that the lack of detectable residue was due probably to its decomposition on or in the soil and/or to its transportation by wind erosion.

Young et al (64) conducted four years of field studies on the persistence of Herbicide Orange and TCDD when applied at massive rates to soils. Herbicide Orange "biodegradation" plots were established in Utah (Air Force Logistics Command Test Range) and in Florida (Eglin AFB Reservation) using simulated subsurface injection techniques to place the herbicide 4 to 5 inches beneath the soil surface in bands 2.5 or 6 inches wide for Utah or Florida, respectively. An application rate of 4,000 lb herbicide/A resulted in initial TCDD residues of approximately 148 ppb and 0.375 ppb in the Utah and Florida plots, respectively. Figure 1 is a semi-logarithmic plot of the soil concentration of Herbicide Orange while Figure 2 is a semi-logarithmic plot of the soil concentration of TCDD in the same field tests. Using Figures 1 and 2, the half-life data were calculated as 300 and 220 days for Orange, and 320 and 230 days for TCDD for Utah and Florida, respectively. It should be emphasized again that these data were from field plots where the herbicide and TCDD were injected as highly concentrated herbicide in narrow bands beneath the soil surface. Data on soil penetration of TCDD within the soil profile of Utah biodegradation plots receiving either 1,000, 2,000 or 4,000 lb/A are shown in Table 1 (Unpublished data: Young, A.L., and E.L. Arnold. 1978. Report on TCDD soil penetration studies. USAF Occupational and Environmental Health Laboratory, Brooks AFB, Texas). Note that in Table 1, 98 percent of all TCDD was detected in the 0-6 inch increment of soil, the increment into which the herbicide was applied. Even in the plots receiving 4,000 lb/A, the TCDD detected in the 6-12

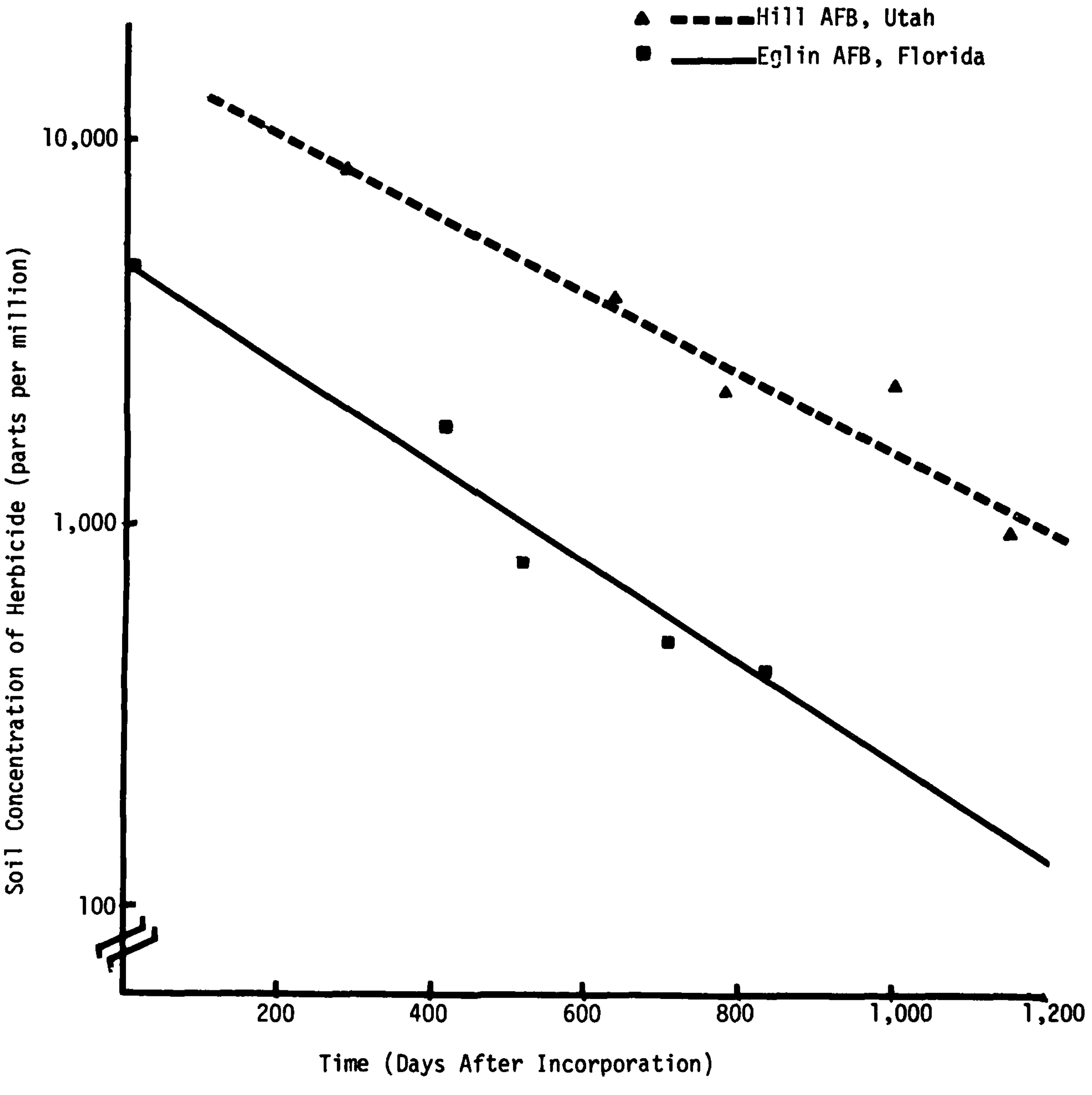

FIGURE 1. Semi-logarithmic plot of soil concentrations (parts per million) of herbicide in Herbicide Orange biodegradation studies at Eglin AFB, Florida, and Hill AFB, Utah. Source: Reference (64).

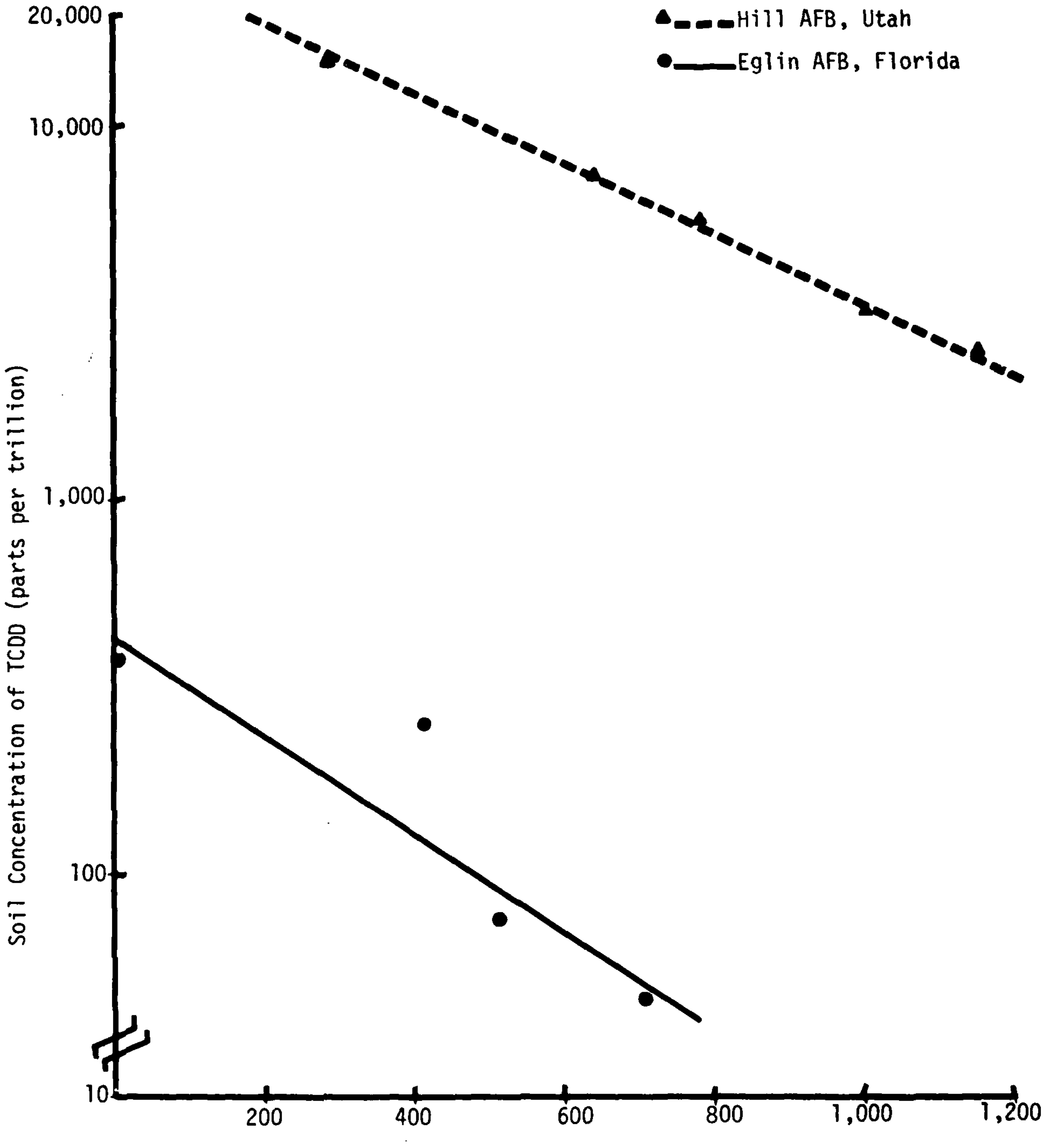

FIGURE 2. Semi-logarithmic plot of soil concentrations (parts per trillion) of TCDD in Herbicide Orange biodegradation studies at Eglin AFB, Florida, and Hill AFB, Utah. Source: Reference (64).

TABLE 1. Concentrations of TCDD, parts per trillion, in the Herbicide Orange biodegradation plots, AFLC Test Range, Utah, four years after applications.[a]

	Original Rate of Herbicide Orange Applied		
Depth (inch)	1,000 lb/A	2,000 lb/A	4,000 lb/A
0 - 6	650	1600	6600
6 - 12	11	90	200
12 - 18	NA[b]	NA[b]	14

[a] Samples collected 6 November 1976. Plots established 5 October 1972.

[b] Samples not analyzed.

Source: Unpublished data (Young, A. L., and E. L. Arnold. 1978. Report on TCDD soil penetration studies. USAF Occupational and Environmental Health Laboratory, Brooks AFB, Texas).

inch increment may have been there because of the mass movement of the herbicide at the time of application rather than through the movement of percolating water. These penetration data are similar to those reported by Young et al (64) for the Florida biodegradation plots (noted earlier) although the Florida site received an annual rainfall of 60 inches (vs 10 inches annual rainfall in Utah).

Young et al (63) reported TCDD data from soil analyses of the Eglin AFB, Florida, Spray Equipment Calibration Grid (Grid 1, Test Area C-52A). As noted in Chapter I, this grid received 1,894 pounds of Purple per acre during the 1962 through 1964 period. TCDD concentrations in a soil profile from samples collected ten years after the last application of Purple are shown in Table 2.

3. Residues in Animals

The current search for TCDD in beef fat and liver in the United States may provide an indication of the possible fate of TCDD in South Vietnam. In September 1974, the Environmental Protection Agency established a Dioxin Implementation Plan which consisted of a short term monitoring program (Part I) and a broad research plan which would take 4 to 5 years to complete (Part II) (3). Part I of the program was initiated in February 1975. The guiding principles for the sample program were: (a) the samples should be representative of beef actually prepared for human consumption and (b) the samples should be from cattle grazed on lands treated with 2,4,5-T. Control samples were to be taken from cattle grazed on non-treated areas within the same state.

Between February and March 1975, 85 beef fat (peritoneal and kidney) and 43 liver samples were collected (3). Approximately 25 percent of these samples were collected from non-treated areas. One laboratory prepared all sample extracts, and identical aliquots were sent to all participating analytical laboratories. In June 1976, analytical results for these samples were announced by the EPA Dioxin Project Manager (53). TCDD was present (range of 20-60 ppt) in a small percentage (3.5 percent) of the beef fat samples taken from cattle with a known exposure of 2,4,5-T. All of the beef liver samples analyzed were negative, at a detection limit of 10 ppt TCDD.

Phase II of the Dioxin Implementation Plan began in 1978, with the intended goal of providing EPA with information on the range and possible bioaccumulation of TCDD in the environment (3). Analyses of human fat and liver tissue and human milk, and additional samples of beef fat and liver were to recieve the highest priority.

Mahle et al (41) have recently completed a surveillance of bovine milk samples from the states of Oklahoma, Arkansas and Missouri. Twenty-five samples were collected from cows grazing on pastures on rangeland treated with normal applications of 2,4,5-T. These samples and control samples were analyzed for TCDD by gas chromatography-mass spectroscopy (GC/MS). They found no TCDD in bovine milk

TABLE 2. Concentration of TCDD in soil profile of Grid 1, Test Area C-52A, Eglin AFB, Florida.[a]

Depth of (inch)	Parts per Trillion (ppt) TCDD
1	150
1 - 2	160
2 - 4	700
4 - 6	44
6 - 36	ND[b]

[a] Grid 1 received 1,894 pounds of Herbicide Purple per acre during 1962-1964. The soil samples were collected and analyzed in 1974.

[b] None detected, minimum detection limit - 10 ppt.

Source: Young et al. (63).

from control or treated areas with a detection limit of 1 ppt.

In reforestation tests in Western Oregon, Newton and Snyder (47) applied Herbicide Orange at the rate of 2-4 lb/A. Analysis of resident mountain beaver captured inside the treated area two months after treatment showed no TCDD in livers, with a minimum detection limit of 3 ppt, and the animals appeared to be in good health in all respects.

Woolson et al (60) examined extracts of 19 bald eagles from locations throughout the United States for TCDD and higher dioxin residues. No dioxins were detected at a minimum detection limit of 50 ppb.

Baughman (7) analyzed samples of human milk for TCDD from areas of South Vietnam heavily treated with 2,4,5-T during the military herbicide program. Levels of 40-50 ppt in human milk were found in samples collected in 1970 and analyzed four years later. Shadoff et al (55) analyzed samples of human milk obtained from mothers residing near the North Concho River Basin of West Texas, an area where large acreages of the watershed had been sprayed repetitively with 2,4,5-T herbicides for brush control over the past 20 years. No TCDD was found in any of the milk samples at a minimum detection limit below 10 ppt.

4. Air Force Studies

Chapter I and earlier sections of this chapter have referenced studies conducted on the Spray Equipment Calibration Grids, Test Area C-52A, Eglin AFB, Florida. The soil residue studies and the aquatic studies have previously been described. Test Area C-52A offered a unique opportunity to follow the fate of TCDD in the many components of the ecosystem. Young (61), Young et al (62, 63, 64), and Bartleson et al (6) have reported on various investigations conducted on this test area. The following is a brief synopsis of the magnitude of the contamination and the subsequent effects upon the wildlife of the test area. In addition to these references, data by Young, Thalken and Harrison (unpublished - USAF Occupational and Environmental Laboratory, Brooks AFB, Texas) of recent investigations at the test site have been incorporated into the synopsis.

Field investigations were conducted during 1973-1978 on the 3.0 km^2 test area containing 4 different calibration grids that received a total of approximately 73,000 kg 2,4,5-T and 77,000 kg 2,4-D during the period 1962-1970. No residues of 2,4,5-T or 2,4-D were detected (detection limit of 10 ppb) in any soil samples collected during 1971-1972. However, residues of the contaminant, TCDD, were still present in 1978.

Fifty-four soil samples were collected to a depth of 0-15 cm from throughout the test area. TCDD levels ranged from <10 to

1,500 parts per trillion (ppt). The median concentration was 30 ppt while the mean was 165 ppt. The ecological survey extending over a five-year period documented the presence of more than a 123 different plant species, 77 bird species, 71 insect families, 20 species of fish, 18 species of reptiles, 18 species of mammals, 12 species of amphibians and 2 species of molluscs. At least 170 biological samples were analyzed for TCDD, including 30 species of animals. No TCDD was found in any of the plant species examined. However, TCDD was found in nine species of animals including two rodent species: beachmice (300-1,500 ppt, liver) and hispid cotton rat (<10-210 ppt, liver); three species of birds: meadowlark (100-1,020 ppt, liver), mourning dove (50 ppt, liver), and Savannah sparrows (69 ppt, liver); three species of fish: spotted sunfish (85 ppt, liver), mosquito fish (12 ppt, whole body), and sailfin shiner (12 ppt, whole body), and one reptile, the six-lined racerunner (360-430 ppt, muscle).

Gross pathology was done on all species collected for TCDD residue analyses. Histopathological examinations were performed on over 300 adult or fetal beachmice or hispid cotton rats from the test area and a control field site. Examinations were performed on the heart, lungs, trachea, salivary glands, thymus, liver, kidneys, stomach, pancreas, adrenals, large and small intestine, spleen, genital organs, bone, bone marrow, skin and brain. Initially, the tissues were examined on a random basis without the knowledge of whether the animal was from a control or test area. All microscopic changes were recorded including those interpreted as minor or insignificant. The tissues were then reexamined on a control and test basis, which demonstrated that the test and control mice could not be distinguished histopathologically. Similar histopathological studies were conducted on the fish and racerunner, and again no significant abnormalities were found.

As a concluding remark, Young et al (64) noted that Test Area C-52A offered a unique opportunity to examine the effects of long-term, low-level exposure of biological systems to TCDD. As previously noted, histopathological examination in body organs from adult and fetal beachmice revealed only lesions which are normally observed in microscopic surveys or large numbers of field animals. The absence of liver lesions in animals that had liver levels of TCDD from 200 to 1,500 ppt was most significant in view of the quantities of TCDD that must have been applied to the test site. Although these pathologic studies were initiated in 1973, beachmice had been collected from the test area as early as 1970 for gross pathological observations. They believed the animals examined in 1973-1974 from Grid 1, the area of greatest contamination (having received 1,894 pounds of Purple per acre in 1962-1924) may have been between 24 and 40 generations removed from the mouse population first noted in 1970. Thus, these studies conducted on the mice of Test Area C-52A suggested that long-term, low-level exposure to TCDD under field conditions may in fact not be teratogenic, mutagenic nor carcinogenic.

D. Environmental Production of TCDD

In 1971, Buu-Hoi et al (14) reported that small quantities of TCDD were formed upon the pyrolysis of 2,4,5-T acid, its butyl ester, or from vegetation defoliated by these products. In their article, Buu-Hoi provided mass spectral data for TCDD (compound I in his text). In reference to these spectral data, they stated (as translated from French):

> There is no need to use the precise analytical techniques described in the foregoing in the case of pyrolysis of 2,4,5-trichlorophenoxyacetic acid (500-600°), because simple fractional sublimation of the pyrolysate, prewashed in diluted aqueous soda, will yield about 5 percent of compound (I). This yield is increased to 15 percent as a result of the pyrolysis of trichloro-2,4,5 sodium phenoxyacetate. The conclusion (and this has been verified) is that quantities of "dioxin" (I) are formed during the combustion, more or less forced, of materials coming from plants pretreated by 2,4,5-trichlorophenoxyacetic acid, and its derivatives (2,4,5-trichlorobutyl phenoxyacetate, the base of the "Orange" defoliant, leads naturally to free acid as a result of hydrolysis attributable to humidity, or to bacterial or fungal degradation), and this is all the more so because alkaline ash appears as a result of such combustion. One then can conceive the possibility of danger, in the long or short term, to public health in areas such as South Vietnam where the people use materials that are principally of plant origin, and are local, as fuels in their homes (wood, charcoal, dry leaves and branches), and which, as a result of the intensive defoliation that took place since 1964, could contain 2,4,5-trichlorophenoxyacetic acid.

In 1972, Saint-Ruf (54), a colleague of Buu-Hoi, reported on the formation of "dioxin" from the pyrolysis of Silvex. He reported that although the quantity of TCDD was less than that observed from the pyrolysis of 2,4,5-T, it was nevertheless sufficiently important to render the use of Silvex extremely dangerous for man and animals, especially in areas where treated vegetable matter was likely to be used as domestic foodstuff.

The data of Buu-Hoi et al (13) and Saint-Ruf (54), and their conclusions, were challenged by Langer et al (38) in 1973. Langer et al investigated conditions which might produce dioxins from salts and esters of 2,4-D, 2,4,5-T and Silvex. No dioxins were detected even when the sodium salts of 2,4,5-T and Silvex were heated to 300°C and

350°C, respectively. However, if a mixture of 0.25 gram (g) 2,4,5-T acid, 2 g H_2O and 10 g k_2CO_3 was refluxed at 100°C for 3 hr, then heated at 200°C for 15 hr, then at 400°C for 43 hr, a total yield of 0.13 percent TCDD could be detected. Furthermore, Langer et al found that the mass spectrum reported by Buu-Hoi et al (and used by Saint-Ruf) for TCDD was in fact not the mass spectrum of TCDD. They suggested that the mass spectrum obtained by Buu-Hoi et al was that of a polymeric matter similar to that found in their own studies. Langer et al concluded that it was extremely unlikely that dioxin (TCDD) could be produced in the field by burning plant material treated with 2,4-D, 2,4,5-T, Silvex or their derivatives.

Recently (1977), Stehl and Lamparski (57) reported finding small amounts of TCDD in trapped residue from self-supported fires of grass and paper treated with different compounds containing 2,4,5-T. Under controlled, but as "natural" as feasible conditions, they analyzed the combustion products of the grass or paper after treatment with 13.3 kg 2,4,5-T per ha (12 lb/A). Stehl and Lamparski felt that the most meaningful way to express their data was in parts per trillion (ppt) of TCDD formed per parts per million (ppm) of 2,4,5-T burned. The average of all their experiments was 0.6 ppt of TCDD formed per 1 ppm of 2,4,5-T burned. The TCDD burden added to the environment by the combustion of natural materials treated with 2,4,5-T would be no larger than 1 ppt of TCDD per 1 ppm of 2,4,5-T residue burned. Ahling et al (1) has reported similar results when 2,4,5-T residue on wood chips is burned at 500°C; 6 ppt of TCDD formed per 1 ppm 2,4,5-T residue burned. They suggested that this would correspond to a formation of about 1 microgram (μg) TCDD per m^2 in forest fire directly after application of the herbicide formulation. However, Cutler (21) recently (1978) has suggested that the burning of forested areas treated with 2,4,5-T may be of little concern, since TCDD decomposes at temperatures above 800°C (and 2,4,5-T decomposes at temperatures above 500°C), considerably below the temperatures of 1200°C or more achieved in the field with a free exchange of air.

E. Photodegradation of TCDD

In perhaps what can be termed as one of the most significant studies on the environmental degradation of TCDD, Crosby and Wong (19), in 1977, found that herbicide formulations (including Orange) containing known amounts of TCDD and exposed to natural sunlight on leaves, soil or grass, lost most or all of the TCDD in a single day, due principally to photochemical dechlorination. Despite the known persistence of pure TCDD, it was not stable as a contaminant in thin herbicide films exposed to outdoor light.

Crosby and Wong (19) have established three requirements for significant dioxin breakdown in the environment; namely, dissolution in a light-transmitting film, the presence of an organic hydrogen-donor such as a solvent or pesticide and ultraviolet light. They noted that all three conditions are normally met during the practical application of 2,4,5-T or other TCDD-containing chemicals. Thus, their data suggested that environmental residues of TCDD often will be considerably less than previously expected.

Nash and Beall (45) concluded from their studies of the fate of TCDD in a microagroecosystem chamber, that once TCDD was volatilized, it dechlorinated in the direct sun and apparently even in shade outdoors or when the sun was filtered with glass in the chambers. They concluded further that TCDD was sensitive to photodechlorination in the vapor phase even in the absence of ultraviolet light.

IV. SUMMARY

Available data indicate that the vast majority of the phenoxy herbicides would impact forest canopy, the intended target. Rapid uptake (e.g., within a few hours) of the ester formulations of 2,4-D and 2,4,5-T would occur. Most of herbicide probably would undergo rapid degradation (weeks) within the cellular matrix of the vegetation. However, some of herbicide may remain unmetabolized and would be deposited on the forest floor at the time of leaf fall. Soil microbial and/or chemical action would likely complete the degradation process.

Herbicide droplets that impacted directly on soil or water would probably hydrolyze rapidly (within hours). Biological and nonbiological degradative processes would further occur to significantly reduce these residues. Some volatilization of the esters of 2,4-D and 2,4,5-T would occur during and immediately after application. The volatile material most likely would dissipate within the foliage of the target area. Photodecomposition of TCDD would minimize the amount of biologically active volatile residues moving downwind of the target area.

Accumulation of phenoxy herbicides in animals may occur following ingestion of treated vegetation. The magnitude of this accumulation would likely be at nontoxic levels. Herbicide residues in animals would rapidly decline after withdrawal from treated feed.

Most TCDD sprayed into the environment during defoliation operations would probably photodegrade within 24 hours of application. Moreover, recent studies suggest that even within the shaded forest canopy, volatilization and subsequent photodecomposition of TCDD would occur. Since translocation into vegetation would be minimal, most TCDD that escaped photodegradation would enter the soil-organic complex on the forest floor following leaf fall. Soil chemical and microbial processes would further reduce TCDD residues. Bioconcentration of the remaining minute levels of TCDD may occur in liver and fat of animals ingesting contaminated vegetation or soil. However, there are no field data available that indicate that the levels of TCDD likely to accumulate in these animals would have a biological effect.

The environmental generation of TCDD from 2,4,5-T residues, through thermal or photolytic processes, would be highly unlikely and of no consequence.

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CHAPTER IV

THE TOXICITY OF 2,4-D, 2,4,5-T AND TCDD IN ANIMALS

I. INTRODUCTION

This review cites a major portion of the world scientific literature dealing with the toxicological aspects of 2,4-D, 2,4,5-T and TCDD in various laboratory and domestic animal species. The primary purpose was to provide a broad overview of research investigations performed to date. With this information, a more critical evaluation could be made of reported human exposures to actual and theoretical levels of 2,4-D, 2,4,5-T and TCDD as presented in other chapters of this report.

In an attempt to organize this chapter the following format and sequence was followed. Each of the compounds in question was reviewed for a) acute and short-term toxicity; b) subacute and chronic toxicity; c) absorption, distribution and excretion data; d) embryotoxic, fetotoxic and teratogenic potentials; e) carcinogenic and tumorigenic potentials; and f) mutagenic and cytogenetic potentials.

Where possible, the cited data were tabularized for ease of comparison and interpretation and a summary of the tabular data presented in the text.

In the review of acute and short-term toxicity the primary effort was directed toward finding references establishing for each compound a no effect dose, a dose lethal to 50 percent (LD_{50}), and a dose lethal to 100 percent (LD_{100}) of the laboratory or domestic animal species studied. After the acute toxic doses were known the subacute and chronic levels were then addressed. The highest "no effect" level of repeated dosing as well as the lowest repeated dosing level causing symptoms of toxicity were collected. These two sections were followed by discussion of cited literature dealing with the absorption, distribution in various body compartments and tissues, and excretion of the 2,4-D, 2,4,5-T and TCDD.

In a 1977 review of the *Teratogenic Effects of Environmental Chemicals* Wilson (145) stated:

> Many chemicals with which man comes into contact are known to be overtly or potentially harmful, causing structural or functional change immediately and these effects are recognized as acute toxic responses to these chemicals. On the other hand, chemicals known to be overtly or potentially harmful, causing structural or functional change and effects, only, after some intermediate time or considerable lapse of time following exposure, are recognized

> as chemicals producing a chronic toxic response. This latter group with its subacute effects is where most of the chemicals fall the at interfere with reproduction. Reproduction effects are rarely the first or only toxic manifestations, but occasionally an embryo or fetus *in utero* is the primary or the only individual expressing the effects of the toxic material, indicating that the conceptus may have extraordinary sensitivity to certain chemicals or compounds. These so called teratogens may be naturally occurring or manufactured materials and despite its sequestered location deep within the maternal body, the embryo or fetus sometimes receives a toxic, i.e., teratogenic dose, albeit only a small fraction of the maternal dose.

Dencker (31) in the introduction to his 1976 study *Tissue Localization of Some Teratogens at Early and Late Gestation Related to Fetal Effects* stated:

> Our knowledge concerning the mechanisms by which chemicals cause fetal damage is very sparse, and only in a few instances have generally accepted theories been presented. Most often there is no specific effect for a given chemical; rather it seems that one agent produces a wide spectrum of malformations - which indicates a nonspecific mechanism of action. Moreover, different chemicals may often produce the same type of malformation. This confusing aspect may partly be explained by the fact that the different organs have certain sensitive periods; in their development, when they are especially susceptible to external influences.

With these comments in mind the literature dealing with embryotoxic, fetotoxic and teratogenic potentials of 2,4-D, 2,4,5-T and TCDD was reviewed to provide as much detail as possible as to the number of animals used, reproductive state, route of administration and toxic response as well as dose and formulation of each compound being tested.

A similar approach was used to review the carcinogenic, tumorigenic, mutagenic and cytogenetic potentials for each of the compounds. Care was taken to try and establish numbers of test animals, method and route of administration and the specific effect of a particular formulation or purity of 2,4-D, 2,4,5-T or TCDD on those animals. Where specific animal data were not available, studies dealing with animal tissue cultures or bacterial mutant strains were referenced.

For simplicity in format, dosage levels of the various chemicals were expressed as mg/kg, but it should be understood that this refers to milligrams of a specific chemical or formulation per kilogram of body weight of the test animal unless otherwise specified.

II. REVIEW OF 2,4-D TOXICITY IN ANIMALS

A. The Acute and Short-Term Toxicity Potentials of 2,4-D

The following review of 2,4-D toxicity was based primarily on three review articles: Rowe and Hyman (115); Dalgaard-Mikkelsen and Poulsen (29); and the International Agency For Research on Cancer (IARC) Monograph, Vol 15 (66) although other recent publications on the subject have been cited.

Bucher (18) in 1946 was among the first to report the results of experiments with small animals using 2,4-D. Temporary myotonia lasting from eight to twenty-four hours or more following a single injection of 150 to 250 mg/kg was observed in mice, rats, rabbits and dogs.

In 1947, Hill and Carlisle (63) published the results of acute oral studies, following single doses of 2,4-D and found the LD_{50} for mice to be 375 mg/kg; for rats, 666 mg/kg; for rabbits, 800 mg/kg; and for guinea pigs, 1,000 mg/kg. The largest single oral dose administered to monkeys without serious after-effect was 214 mg/kg or 428 mg/kg given intraperitoneally. An oral, plus an intraperitoneal injection in monkeys for a total dose of 500 mg/kg 2,4-D caused nausea, vomiting, lethargy, muscle incoordination and head drop. These workers observed that all species reacted similarly and that there were no significant differences in potency between crude and purified preparations, or between the sodium or ammonium salts. Deaths from large doses were apparently due to ventricular fibrillation. When death was delayed, myotonia, stiffness of extremities, ataxia, paralysis and coma were observed.

Parenteral administration of 150-200 mg/kg 2,4-D caused symptoms of myotonia in mice. In those acutely intoxicated, dilatation of the blood vessels of lungs, liver and kidneys was observed by Bucher (18).

Rats and guinea pigs administered lethal doses of 2,4-D exhibited congestion of the viscera. Enlarged, swollen, kidneys and microscopically massive cloudy swelling of the proximal convoluted tubules with cast formation was noted by Hill and Carlisle (63).

Florsheim and Velcoff (43) reported a decrease in both thyroid and body weights in male rats given single subcutaneous injections of 2,4-D at 100 mg/kg.

Guseva (57) found the LD_{50} for the subcutaneous injection of 2,4-D in mice to be 220 mg/kg. At 10-100 mg/kg 2,4-D in rats and mice no impairment of motor activity was seen nor did those doses alleviate strychnine spasms. In cats, 20-30 mg/kg 2,4-D given intravenously was hypotensive and that effect was not impaired by atropinization.

Baker et al (6) administered 112 grams (g) of grass mixed with horsemeat and dog meal divided over three consecutive meals to two healthy one-year-old mongrel dogs. The grass had been treated two days earlier with the equivalent of 4 pounds per acre (lb/A) of 2,4-D butyl ester, which was twice the recommended rate. The dogs readily ate the food and no ill effects were observed during the following 96 hours. Each animal was then treated with 500 mg/kg 2,4-D in a single oral dose. No deleterious effects were seen in the next 96 hours of observation. One animal, killed and necropsied at 96 hours post administration, failed to reveal any macroscopic lesions and the other animal remained healthy for 82 days following the second treatment, at which time the experiment was terminated.

In dogs, Drill and Hiratzka (33) found that toxic symptoms were often delayed up to six hours following a single oral administration of lethal doses of 100, 250 and 400 mg/kg 2,4-D. The deaths were delayed and occurred two to nine days after the compounds were administered. The acute oral LD_{50} for (98.5 percent purity) 2,4-D was in the range of 100 mg/kg or higher. Death appeared to be due in most cases to hepatic congestion or pneumonia. Pathological changes were limited to the gastrointestinal tract, lungs and liver, and followed the development of anorexia, weight loss and myotonia (33). Dogs exhibited more evidence of hepatic congestion and moderate hepatic necrosis than was seen in other animals studied by Bucher (18). Drill and Hiratzka (33) concluded that the no effect level for a single oral dose of 2,4-D in dogs was 25 mg/kg.

In a study by Shavgulidze et al (125), the single oral LD_{100} of 2,4-D sodium salt in sheep was 900 mg/kg. Death occurred in 2-4 days following the clinical signs of asthenia, depression, ataxia, hypothermai, dyspnea, muscle paralysis, anorexia and intense photophobia in those animals dosed at 500-1,000 mg/kg. The no effect single, oral dose of 300-400 mg 2,4-D was detoxified in 9-12 days with no traces remaining in the tissues.

McLennan (88), reported on the accidental oral administration of 2,4-D in two cows. He noted that the death of one animal occurred within 12 hours following a calculated dose of 150-188 mg/kg. The toxic dose for the animal that survived, was calculated at between 105 and 132 mg/kg. In contrast Rowe and Hymas (115) noted that the LD_{50} of 2,4-D for cattle ranged between 500 and 2000 mg/kg body weight while a single dose of 1000 mg/kg may or may not cause illness. Radeleff (110) cited a report in which cattle given one dose of 250 mg/kg 2,4-D showed signs of toxicity. In calves six to eight weeks old, Bjorklund and Erne (15) found that single doses of 100 to 200 mg/kg 2,4-D produced reversible signs of toxicity.

Toxic symptoms summarized by Rowe and Hymas (115) included the following general observations in animals treated with acute toxic doses of 2,4-D: loss of appetite, loss of weight, depression, roughness of coat, general tenseness and muscular weakness particularly of the posterior quarters. Post mortem findings usually included irritation of the stomach of small animals and of the abomasum of ruminants, minor evidence of liver and kidney injury and in some instances congestion of the lungs.

From data presented in Table 1, the acute LD_{50} as a rule was in the order of 300-800 mg/kg 2,4-D for rats, mice, guinea pigs, rabbits and cats. Analyses of data from the limited available studies supported the conclusion that the dog may be slightly more susceptible to oral doses of 2,4-D than other animals. Monkeys, sheep and cattle appeared to be somewhat more tolerant. The experiments referenced in Table 1 have also provided information on the acute oral administration of various salts and esters of 2,4-D as pure chemicals and as commercial preparations. No significant differences in the toxicity of the salt and ester forms of 2,4-D were seen when compared to the free acid (63,115).

Hill and Carlisle (63) stated:

> In any assessment of the acute toxicity of a chemical based on data obtained from laboratory animals it should be borne in mind that considerable variation in species susceptibility may occur and that the data obtained cannot always be translated into toxic doses for humans.

In the studies referenced in this section it can be concluded as Hill and Carlisle did in their studies that:

> all of the laboratory animals tested reacted in a similar fashion from signs and symptoms which developed and from the pathological lesions which were present at autopsy. Assuming that man is no more resistant or susceptible than the rabbit or monkey, then the largest tolerated dose for a 75 kg man would be 15 grams of 2,4-D.

With the exception of dog and monkey, all of the laboratory animals used in the cited references lacked the vomiting reflex so that they were unable to relieve themselves of irritating material by vomiting.

> The experiments conducted in monkeys indicate that the material is a gastric irritant in large doses, so that the possibility of the occurrence of acute poisoning in humans would seem relatively remote because of the large dose which man could presumably tolerate. Assuming that man is no more susceptible than the most susceptible animal test, the mouse, then the calculated oral LD_{50} for man would amount to approximately 28 grams (63).

It is generally accepted that the oral LD_{50} of 2,4-D for man is around 500 mg/kg while the accepted LD_{50} of aspirin for man is around 1,500 mg/kg.

B. The Subacute and Chronic Toxicity Potentials of 2,4-D

Repeated once or twice daily, subcutaneous injections of 50 to 90 mg/kg 2,4-D in mice for three weeks to ninety days did not elicit a characteristic chronic syndrome of toxicity or any notable histological

TABLE 1 . Summary of literature data on the no-effect, LD50 and LD_{100} levels of the acute toxicity of 2,4-D in animals

Animal	Number Used	Route of Administration	Dose-Toxicity	Single Dose mg/kg	Reference
Mouse					
	450	Gavage	LD_{50}	375[a,b]	63
	NS[c]	Intraperitoneal	LD_{50}	375[a]	63
	NS	Subcutaneous	LD_{50}	220[a]	57
	NS	Subcutaneous	LD_{50}	280[b]	18
	NS	Oral in olive oil	LD_{50}	368[a]	115
	NS	Oral in olive oil	LD_{50}	541[d]	115
	NS	Oral in corn oil	LD_{50}	713[e]	115
	NS	Oral	LD_{50}	380[f]	81
Rat					
	150	Gavage	LD_{50}	666[b]	63
	NS	Intraperitoneal	LD_{50}	666[b]	63
	NS	Oral in water	LD_{50}	805[b]	115
	NS	Oral in olive oil	LD_{50}	375[a]	115
	NS	Oral in olive oil	LD_{50}	700[d]	115
	NS	Oral in corn oil	LD_{50}	620[e]	115
	NS	Oral	LD_{50}	1,500[f]	119
	NS	Oral	LD_{50}	2,000[b]	119
	NS	Oral	LD_{50}	900[f]	81
Guinea Pig					
	125	Gavage	LD_{50}	1,000[b]	63
	NS	Intraperitoneal	LD_{50}	666[b]	63
	NS	Oral in water	LD_{50}	551-2000[b]	115
	NS	Oral in olive oil	LD_{50}	469[a]	115

Table 1 continued

	NS	Oral in olive oil	LD_{50}	550[d]	115
	NS	Oral in corn oil	LD_{50}	848[e]	115
Rabbit	70	Gavage	LD_{50}	800[b]	63
	NS	Intraperitoneal	LD_{50}	400[b]	63
	NS	Intravenous	LD_{50}	400[b]	63
	NS	Oral in corn oil	LD_{50}	424[e]	115
Cat	NS	Oral	LD_{50}	820[f]	81
Dog	4 F[g]	Oral	LD_{50}	100[a]	33
	1 M[h]/2 F	Oral	No effect	25[a]	33
	2	Oral	No effect	500[f]	6
Monkey	1	Oral	No effect	214[a]	63
		Intraperitoneal	No effect	428[b]	63
Sheep	NS	Oral	LD_{100}	900[b]	125
			No effect	300-400[b]	125
Cattle	1	Oral	LD_{100}	150-188[i] (a calculated dose)	88
		Oral	LD_{50}	500-2000[j]	115,132

[a] 2,4-D acid

[b] Sodium salt of 2,4-D

[c] NS - number of animals in study not stated or unavailable from literature source.

[d] Isopropyl ester of 2,4-D

[e] Mixed butyl esters of 2,4-D

Table 1 continued

[f] Butyl ester calculated as 2,4-D

[g] F - Female

[h] M - Male

[i] 20% w/v amine salt of 2,4-D in aqueous solution

[j] Form not stated in available literature source

changes. Levels of 70 mg/kg or more retarded growth, probably by reducing food intake. Mice undergoing this treatment became pregnant and bore apparently normal litters (18).

Guseva (57) found that 22 daily subcutaneous injections of 0.1 mg 2,4-D in mice caused no toxic effects.

In subacute studies with rats, Hill and Carlisle (63) fed a diet containing 1,000 mg 2,4-D/kg for 30 days without severe harmful effects. Some visceral congestion and kidney edema with degenerative changes in the tubules were noted.

No adverse effects were seen in groups of 5 or 6 young female rats given 2,4-D by intubation five times a week for four weeks at doses of 3, 10, and 30 mg/kg in olive oil. At doses of 100 mg/kg 2,4-D, varying degrees of gastrointestinal irritation, slight cloudy swelling in the liver and depressed growth rates were noted. At 300 mg/kg 2,4-D, the animals failed rapidly and died. The principle lesion observed at post mortem was a severe gastrointestinal irritation. In another study, matched groups of five, young, adult, female rats were placed on diets containing 100, 300, 1,000, 3,000 and 10,000 mg 2,4-D/kg of diet for 113 days. The no effect levels were 100 and 300 mg/kg of diet. At 1000 mg/kg, adverse effects were characterized by a depressed growth rate, excessive mortality, slightly increased liver weights and slight cloudy swelling of the liver. The animals on the 3,000 and 10,000 mg/kg levels in their diets were destroyed after twelve days as they were not eating and were rapidly losing weight. Increased liver and kidney weights were noted with unstated minimal pathological changes (115).

No drastic damaging effects were noted when male Long-Evans rats were given 2,4-D equivalent to 2-5 g/kg over a 4 to 7-week feeding period. Response to herbicide treatment was dependent on animal age and on the duration of time that the chemical was fed. Little or no effect was noted on liver weight. Herbicide-induced enlargement of the liver was associated with increases in most of the major cellular components on a per liver basis. Isolated liver nuclei were 20-30 percent more active in the *in-vitro* RNA synthesis than in the control nuclei (22).

Schwetz et al (122) found that oral doses of 12.5 to 87.5 mg/kg 2,4-D did not adversely affect the weight gain of rats during pregnancy. In a preliminary study, non-pregnant rats tolerated 75 mg/kg 2,4-D for 10 days while 100 mg/kg killed two rats and produced overt signs of toxicity in three survivors.

Hansen et al (58) conducted a study with rats starting at 3 weeks of age, using groups of 25 female and 25 male animals, fed 0, 5, 25, 125, 625 or 1,250 mg 2,4-D/kg of diet for 2 years. During the study no significant differences in survival rates between controls and test animals were noted. The mean body weights of the different groups of males and females and the organ-to-body weight ratios for liver, kidney, heart, spleen and testes were not significantly different (P>0.025). The only exceptions were in two male rats, one at 625 mg/kg, and a second at 125 mg/kg dosage level in the diet. These two animals had slightly enlarged spleens. Mean values for hemoglobin, hematocrit, and total white blood cell count of controls and of rats at each dose level, at the same time interval, were similar and within normal range. The maximum no effect level for the rats in this study was greater than 1,250 mg 2,4-D/kg of diet.

Hansen et al (58) in another study fed 0, 100, 500 or 1,500 mg 2,4-D/kg of diet to groups of 20 male and 20 female rats. No effect was observed at the 100 and 500 mg/kg of diet levels. At the 1,500 mg/kg level, there was no effect on fertility nor on the average number of pups per litter; however, significant effects on the average number of pups weaned and also on their weaning weights were noted. The no effect level is at least 500 mg/kg but less than 1,500 mg/kg of 2,4-D in the diet.

Bjorklund and Erne (15) administered 2,4-D in drinking water to rats at 1,000 mg/l. Progeny from treated females were maintained on the same dose level for 2 years with signs of growth inhibition, poor general health and diarrhea as the main effects.

Kay et al (72) found no significant adverse effects in a study using 112 New Zealand strain albino rabbits where 15 ml of each of three commercially available formulations of 2,4-D (dimethylamine salt and the isooctyl and butyl esters) were administered 5 times a week for 3 weeks to the intact and abraded skin at 0.626 percent and 3.13 percent concentrations. Body weights, survival, hematological values, clinical chemistry values and organ/body weight ratios were all within normal ranges. Local skin inflammatory reactions occurred in all groups of animals including controls. This was especially severe in those applications where the 2,4-D esters were diluted with an unspecified oil. The water dilutions of all three forms produced less local skin inflammation. Histologically, the treated animals had an increased incidence and severity of subepithelial fibrosis and accompanying mononuclear infiltration in the skin. No peripheral or central nervous system tissues or microsections of other tissues disclosed any adverse findings.

Hansen et al (58) conducted a study using groups of 3 male and 3 female beagle dogs being fed 0, 10, 50, 100 or 500 mg/kg 2,4-D in the diet (96.7 percent pure, with no detectable TCDD by GLC with a sensitivity of 1 mg/kg) for 2 years, starting at 6-8 months of age. Twenty-eight dogs surviving the 2-year period were clinically normal, in fair to good condition, with a no effect level greater than 500 mg/kg in the diet. One female at the 100 mg/kg level was emaciated at the end of the experiment; however, no significant lesions were noted. A male animal that died after 10 months on the study at the 10 mg/kg 2,4-D level showed a slight atrophy of the testes and moderate depletion of cellular elements in other tissues.

Drill and Hiratzka (33) orally dosed (via capsule in a piece of canned dog food) adult mongrel dogs of both sexes with either 2, 5 or 10 mg/kg 2,4-D 5 days a week for 13 weeks. The 2,4-D was a commercial product of 98.5 percent purity (label stated). All dogs survived this study and no significant symptoms of toxicity were seen and no changes in body weight, organ weights, or blood count were noted. In a separate study, three of four dogs given daily doses of 20 mg/kg 2,4-D died between days 18 and 49. The signs observed in these animals differed somewhat from those seen in the acute studies. The chronically treated animals displayed stiffness of hindlegs and ataxia, weakness, difficulty in chewing and swallowing and occasionally bleeding from the gums. Weight loss occurred after 7-12 days and a terminal fall in lymphocyte count occurred prior to death. The authors stated, "Death during the repeated administration of 2,4-D was not related to pathological changes in the liver, kidneys, or other organs examined."

Seabury (123) treated three dogs experimentally infected with histoplasmosis, by intravenous injections of sodium 2,4-D at the rate of 1.17, 2.6, and 3.2 mg/kg per injection for 32-37 days without evidence chronic toxicity.

Bjorklund and Erne (15) treated young pigs at varying intervals up to 103 days with 50, 100 or 300 mg/kg of the commercial triethanolamine salt or butyl ester of 2,4-D. Exhibited symptoms of intoxication and pathology were analagous to those seen in laboratory animals. Clinical signs of anorexia and retarded growth were found in one animal given 51 doses of 50 mg/kg triethanolamine salt over 103 days. Pigs fed 500 mg/kg of diet triethanolamine salt of 2,4-D for up to 12 months developed locomotor disturbances of increasing severity after about one month. Animals sacrificed after 2-12 months had normal organ weights and no gross pathological changes. Clinical chemistry observations included lowered hemoglobin and hematocrit values, elevation of glutamic-oxaloacetic transaminase and reduced albumin and albumin: globulin ratios in the treated animals [see IARC Monograph, Vol 15 (66)].

Shavgulidze (125) observed transient hematological changes in sheep receiving daily doses of 18 mg/kg 2,4-D sodium salt for 120 days.

Mitchell et al (90) fed a cow 5.5 g of 2,4-D acid daily for 106 days with no apparent harmful effects on the health or milking performance. Post mortem examinations revealed no pathological changes in the liver, kidneys or body fat. By biological assay the presence of 2,4-D was demonstrated in the blood serum; however, 2,4-D was not found to be secreted in the milk nor was it found in the blood serum of a calf fed milk from this cow.

Palmer (99) found that yearling steers needed to be given 15 daily doses of 250 mg/kg of the alkanolamine salt of 2,4-D before signs of toxicity occurred. He found that 112 daily doses of 50 mg/kg of this 2,4-D salt had no deleterious effect on the steers.

Rowe and Hymas (115) in reviewing the chronic toxicity of 2,4-D stated:

> The results of repeated oral administrations indicate that 2,4-D can be tolerated without adverse effects in doses only slightly smaller than those which cause toxic effects when given only once. This fact demonstrates that 2,4-D has a low degree of chronic toxicity.

The same general observations of toxicity were noted in animals receiving chronic toxic doses of 2,4-D, as were seen in animals given single toxic doses. These were loss of appetite, loss of weight, depression, roughness of coat, general tenseness, and muscular weakness particularly of the posterior quarters. Post mortem findings usually included irritation of the stomach and gastrointestinal tract of small animals and abomasum of ruminants with only minor evidence of gross and histopathological injury in the liver and kidneys.

Study of the data presented in Table 2 indicated that mice tolerate subcutaneous injections of 2,4-D at 50-70 mg/kg with no effect, while 70-90 mg/kg retards growth. Rats tolerated 1,000-1,250 mg/kg 2,4-D in their diet and 75 mg/kg orally without toxic effects. At levels of 1,000 mg/kg 2,4-D in the water and 1,500 mg/kg in the diet and 100 mg/kg orally, toxic signs were noted. Rabbits showed no gross differences between test and control animals, as far as skin irritation, when 3.13 percent solutions of various formulations of 2,4-D were placed on their intact or abraded skin. Dogs tolerated 500 mg/kg diet or 10 mg/kg orally with no toxic signs, while 20 mg/kg caused death in three of four animals. Oral doses of 300 to 500 mg 2,4-D/kg of diet were toxic to pigs. Rowe and Hymas (115) stated:

> Cattle demonstrate a similar susceptibility to 2,4-D as do the small laboratory animals. Cattle are distinctly more tolerant of 2,4-D than are dogs. Cattle can probably tolerate 30-50 mg/kg/day [(99)] for long periods without adverse effects. Daily doses of 100-250 mg/kg (99) would have to be continued for a week or longer to cause ill effects in cattle. A single dose of 500-1,000 mg/kg is not likely to cause problems; however, if repeated, serious effects and deaths are likely to occur.

The chronic toxicity of 2,4-D did not differ greatly from the acute toxicity. At only slightly lower doses the same general signs, symptoms, and pathology were seen.

Hansen et al (58) made the following statement on chronic exposure of humans to 2,4-D residues. (The cited values were for 1971. They have now been lowered slightly; however, in this case they were used to establish a worst-case situation.)

TABLE 2 . Summary of literature data on the subacute and chronic toxicity of 2,4-D in animals

Animal	No. Used	Route of Administration	Effect	Dose	Reference
Mouse					
	NS[a]	1-2 daily s.c. injections for 3 weeks to 90 days	No effect	50-70 mg/kg[b]	18
	NS	1-2 daily s.c. injections	Retarded growth	70-90 mg/kg[b]	18
	NS	22 daily s.c. injections	No effect	0.1 mg/inj[c]	57
Rat					
	NS	30 days in diet	No severe effect	1000 mg/kg diet[b]	63
	6 F[d]	5 doses/wk for 4 wks by intubation	No effect	30 mg/kg[e]	115
	6 F	5 doses/wk for 4 wks by intubation	Liver, G.I. growth effect	100 mg/kg[e]	115
	6 F	5 doses/wk for 4 wks by intubation	Fatal in days	300 mg/kg[e]	115
	5 F	113 days in diet	No effect	300 mg/kg diet[e]	115
	5 F	113 days in diet	Liver and growth effects, deaths	1000 mg/ kg diet[e]	115
	44 M[f]	4 or 7 wks in diet	Slight effect	Total 2-5 g/kg[c]	22
	5 F	10 daily doses via stomach tube	No effect	75 mg/kg[c]	122

Table 2 contunied

	5 F	10 daily doses via stomach tube	2 died, overt toxicity in 3	100 mg/kg[c]	122
	25 F/25 M	In diet for 2 yrs	No effect	1250 mg/ kg diet[c]	58
	20 F/20 M	In diet for 3 generation reproduction study in adults	No effect	500 mg/kg diet[c]	58
	20 F/20 M	In diet for 3 generation reproduction study in adults	No effect on fertility or litter size. Lower no. pups weaned, lowered weight	1500 mg/kg diet[c]	58
	NS	In drinking water for 2 yrs.	growth inhibition, poor health, diarrhea	1000 mg/ 1 water[b]	15
Rabbit	22 F/22 M	5 times/wk for 3 wks to intact and abraded skin	No effect at gross exam. Some histopath effects in 2,4-D/oil treated animals.	3.13% solution[g]	72
Dog	3 F/3 M	In diet for 2 yrs	No effect	500 mg/kg diet[c]	58
	3 M	Oral dose via capsule 5 days/wk for 13 wks	No effect on gross	10 mg/kg[c]	33
	1 F/3 M	Oral dose via capsule 5 days/wk for 13 wks	Death in 3 at 18-49 days. Severe signs in 1 animal surviving	20 mg/kg[c]	33

Table 2 continued

	3 F	Daily I.V. doses for 32 days at two higher levels, 37 days at lower level	No effect	1.1 mg/kg[b] 2.6 mg/kg[b] 3.2 mg/kg[b]	123 123 123
Pig					
	NS	Oral doses up to 103 days	Toxicity, anorexia, retarded growth	300 mg/kg[h]	15
	NS	In diet up to 12 months	Locomotor problems, normal organ weights, no gross pathology	500 mg/kg[i]	15
Sheep					
	NS	Daily oral doses for 120 days	Transient hematological and biochemical changes	18 mg/kg[b]	125
Cattle					
	1 F/L[j]	Daily oral dose for 106 days	No effect	5.5 g[c]	90
	NS, S[k]	15 daily oral doses	Toxicity	250 mg/kg[i]	99
	NS, S	112 daily oral doses	No effect	50 mg/kg[i]	99

[a] NS - number of animals in study not stated or unavailable from literature source

[b] Sodium salt of 2,4-D

[c] 2,4-D acid

[d] F - Female

[e] Butyl ester calculated as 2,4-D

[f] M - Male

[g] Sodium salt, isooctyl ester and butyl ester of 2,4-D each applied separately on individual animals under the conditions described and concentration listed.

[h] Amine salt and butyl ester of 2,4-D used separately in animals at dose indicated.

[i] Amine salt

[j] F/L, Female lactating

[k] S - Steer

> Adequate data are not available to enable one to state conclusively what the total level of 2,4-D residues may be in foods ingested by the human population. An estimate of the greatest amount that might possibly be ingested can be made by use of the legal tolerances established by the FDA for 2,4-D in various crops. They are 5 mg/kg on 4 fruit crops (apples, citrus fruits, pears and quinces) and 0.5 mg/kg on 4 grain crops (barley, oats, rye and wheat). If it is assumed that all the crops for which a tolerance exists always carried the maximum amount of 2,4-D permitted, it can be calculated that approximately 0.3 mg/kg of 2,4-D would be contributed to the total diet (fruit crops = 6 percent of the dietary intake of man and grain crops = 9 percent). When the maximum estimated human exposure to 2,4-D via the diet is compared to the dosages given rats in the present study, it is apparent that there is an extremely wide margin of safety between 0.3 mg/kg of diet in man and the 1,250 mg/kg of diet fed to rats.

C. Absorption, Distribution and Excretion of 2,4-D

Different degrees of sodium 2,4-D poisoning were produced by Elo and Ylitalo (35) in adult male Sprague-Dawley rats when 250 mg/kg 2,4-D was administered by subcutaneous injection. After various intervals the concentration of intravenous ^{14}C-2,4-D was compared to the level found in the cerebrospinal fluid (CSF) and brain. At 4.5 hours when the sodium 2,4-D radioactivity in plasma had diminished to 67 percent of control levels, an 11-fold increase in the brain and a 39-fold increase in the CSF were seen compared to a 4.5 fold increase in the liver. All physiological and toxicological parameters of this 1977 study had not been fully analyzed; however, during acute 2,4-D poisoning, the levels found in the brain were greatly increased. This increase appeared to be closely associated with the toxic symptoms.

In rats, pigs and calves, 2,4-D administered in doses of 50-100 mg/kg orally as salts were readily absorbed and eliminated, mainly in the urine, with plasma half-lives varying from 3-12 hours (38, 39). The rate of 2,4-D elimination in rats was dosage dependent. Following administration of ^{14}C-2,4-D, Khanna and Fang (73) found radioactivity in all organs and tissues examined [see IARC monograph, Vol 15 (66)].

Berndt and Koschier (9) using ^{14}C-labeled 2,4-D in rat and rabbit renal cortical tissue slices, *in vitro*, noted that 2,4-D was transported by the classical renal organic anion transport process; however, other mechanisms of transport may also have been involved. This study may help explain one of the mechanisms contributing to the relatively rapid disappearance of 2,4-D from most species and the low levels of biological deposition of this compound.

The esters of 2,4-D were hydrolyzed in animals and the phenoxy acids were excreted predominantly as such in the urine of rats after oral administration, although a minor portion of them may have been conjugated with the amino acids glycine and taurine and with glucuronic acid (54). No 2,4-dichlorophenol was detected, however, in the urine of C57BL/6 mice treated subcutaneously with 2,4-D or its butyl or isooctyl esters. The rates of disappearance from plasma of 2,4-D and its butyl and isooctyl esters following single subcutaneous injections of 100 mg/kg of the compounds to female C57BL/6 mice were: butyl ester > isooctyl ester > 2,4-D (147), [see IARC monograph, Vol 15 (66)].

After oral administration of 0.05 mg/kg 2,4-D to rats, Fedorova and Belova (42) found that traces were detected in the milk of lactating animals for six days. Within 24 hours after administration of 2,4-D to pregnant rats, 16.8 percent of the dose was detected in the uterus, placenta, fetus and amniotic fluids, [see IARC monograph, Vol 15 (66)].

Bjorklund and Erne (15) found that 2,4-D passed the placental barrier in pigs.

Clark et al (23) fed 2,4-D acid (99 percent purity) to groups of 3, adult beef cattle and adult sheep at levels of 0, 300, 1,000 and 2,000 mg/kg of feed for 28 days. Animals were killed and tissues sampled one day after the last dose, others one week later. Residues of the 2,4-D and its phenol metabolites were determined in muscle, fat, liver and kidney. Muscle and fat contained the lowest levels while kidneys and liver contained the highest residue level. Withdrawal from treatment for one week before killing resulted in a significant reduction in tissue residue levels. With the exception of the kidneys, 2,4-D residues averages less than 1 mg/kg in the tissue analyzed. The kidney tissue level averaged 7.82 mg/kg with 0.37 mg/kg present after a 7-day withdrawal period. No 2,4-D was detected in fat or muscle of any animals at a detection limit of 0.05 mg/kg. All treated animals showed some anorexia, weight loss or poor weight gain depending on the level 2,4-D present in the feed, due to lowered palatability. During the 7-day withdrawal period, feed consumption in all groups returned to normal.

2,4-D was rapidly eliminated from animals, mainly in the urine with plasma half-lives of 3-12 hours following a single dose. Generally, it accumulated in animal tissues when given at high doses or repeated lower doses. However, these residues declined rapidly with a half-life of 1 to 2 weeks. Because of its excretion by the kidney, kidney tissue levels were as much as twenty times greater than the level seen in other organs and tissues.

D. Embryotoxic, Fetotoxic and Teratogenic Potentials of 2,4-D

The embryotoxic, fetotoxic and teratogenic potentials of 2,4-D appeared to be extremely variable with observable effects dependent upon concentration, degree of purity and method of administration with some effects only occurring with doses that approached maternal toxicity.

Bionetics Research Laboratories (12, 13, 14) reported that either the acid, or the isopropyl, butyl and isooctyl esters of 2,4-D, administered orally or subcutaneously at days 6-14 of gestation, increased the incidence of anomalous fetuses among BL6, AKR and C3H strains of mice but not among B6AK and A1Ha strains. No single strain showed a positive response to all formulations. No single formulation caused a positive response among all strains of mice. Thus, the reported effects were highly strain-specific. In addition, the Bionetics study involved parenteral administration using dimethyl sulphoxide (DMSO) as a vehicle, which complicated the interpretation of the data, since DMSO has been shown to be a teratogen in several species of laboratory animals when administered by the route used in the Bionetics study [see Schwetz et al (122)].

Schiller (118) found no difference in fertility (defined as the number of rats weaned per female mated) of test and control animals in one experiment where rats were fed potatoes which had been treated with 2,4-D. In a combined second and third experiment, fertility of the P, F_1, F_2 and F_3 generations was 7.2, 5.8, 6.8 and 6.1 for controls versus 7.2, 7.1, 5.4 and 5.1, respectively, for test rats. The differences between control and test groups were not significant ($P>0.05$). The content of 2,4-D, its form, or purity in the potatoes was not given.

When 1,000 mg/1 2,4-D was given throughout pregnancy to Sprague-Dawley rats via the drinking water, the gestation and parturition were normal. The litter size was not significantly reduced and no anomalies were seen in the pups (15).

Hansen et al (58) stated that in unpublished work performed by T.B. Gaines and R.D. Kimbrough, female rats were fed 2,4-D acid at 0, 1,000, and 2,000 mg/kg of diet for 95 days, mated with untreated males and continued on their respective diets throughout gestation and lactation. At the highest dosage level, females gave birth to pups that were small at birth and 94 percent died before weaning. Some deaths also occurred in pups of females fed the lower level.

Starting with rats three weeks of age, groups of 25 female and 25 male animals were fed for two years either 0, 5, 25, 125, 625 or 1,250 mg 2,4-D/kg of diet. No significant effects on growth rate, survival rate, organ weights or hematologic values were noted (58). Hansen et al (58) also noted in a three generation, six litter rat reproduction study, no deleterious effect of dietary 2,4-D acid at 100 or 500 mg/kg was evident. At 1,500 mg/kg, however, 2,4-D, while apparently affecting neither fertility of either six nor litter size, sharply reduced the percent of pups born surviving to weaning and the weights of weanlings.

In studies by Schwetz et al (122), the acid of 2,4-D, the propylene glycol butyl ether ester of 2,4-D and the isooctyl ester of 2,4-D were evaluated for effects on fetal development, neonatal growth and survival when administered at 12.5, 25, 50, 75 and 87.5 mg/kg orally to pregnant Sprague-Dawley (Spartan strain) rats during organogenesis (days 6-15 of gestation). Fetuses were delivered by Caesarean section on day 20 of gestation and were examined grossly, measured and weighed. Fetotoxic responses seen at high dose levels 50, 75 and 87.5 mg/kg included subcutaneous edema, delayed ossification and wavy ribs. Teratogenic responses were not seen at any dose level. 2,4-D did not affect fertility, gestation, lactation or viability of the newborn. The esters of 2,4-D decreased viability of the newborn and lowered lactation indices (Lactation Index: pups weaned/pups alive on day 4 X 100). In a second part of the experiment in which litters delivered naturally, 2,4-D and its esters had little or no effect on fertility, gestation, viability or lactation indices. There were no observable effects on neonatal growth and development.

Khera and McKinley (74) observed minimal 2,4-D induced fetopathy and an increased incidence of skeletal anomalies in rat pups following single daily oral doses of 100-150 mg/kg 2,4-D from days 6 to 15 of gestation. The observed skeletal defects did not appear to be incompatible with postnatal survival. Following treatment of dams with the acid of 2,4-D and the butyl and isooctyl esters of 2,4-D, weight gain and viability of the offspring were within control limits. The findings of their study suggested that postnatal parameters were unrelated to the teratologic potential of the chemicals.

No consistent embryotoxic effects were noted when 2,4-D acid was administered orally to hamsters at doses of up to 100 mg/kg on days 6-10 of gestation (24).

Binns and Johnson (10) showed that 2,4-D did not have a teratogenic potential in sheep. Starting one day after breeding ewes were given 2 g/day of 2,4-D acid in an alfalfa meal/water mixture via stomach tube for 30, 60, or 90 days. No clinical signs of toxicity nor histopathologic lesions were seen in the ewes and no congenital anomalies nor histopathologic lesions were seen in the lambs.

Ewes were reported to have had increased rates of stillbirths and bucks displayed reduced sexual activity and decreased sperm quality when pastures were grazed soon after treatment with 3 lb/A of the 2,4-dichlorophenoxybutric acid (2,4-DB) (116).

When a diet containing 500 mg/kg 2,4-D was fed to a sow during the entire pregnancy, the sow was anorexic and the newborn piglets were underdeveloped and apathetic with 10/15 dying within 24 hours. When the survivors were continually fed 2,4-D at 500 mg/kg of diet until they were 8 months of age marked growth depression, persistent anemia and moderate degenerative changes of the liver and kidneys were noted (15).

Erne (40) fed pregnant reindeer birch leaves that had been sprayed with a mixture of 2,4-D and 2,4,5-T at a daily dose of 1 mg phenoxy herbicide per kg body weight. There were no clinical or histopathological changes noted in any of the female reindeer and no fetal anomalies were seen.

From the data presented in Table 3 the no effect level for embryotoxic, fetotoxic and teratogenic signs in the rat was approximately 1,000 mg/l of the sodium salt of 2,4-D, while the no effect level from 2,4-D acid in the rat diet ranges from 1,250 to 1,500 mg/kg of food. Oral doses of 2,4-D acid and the butyl and isooctyl esters cause no effect at daily doses of 87.5 mg/kg. At 100 to 150 mg/kg 2,4-D acid and esters produced embryo and fetotoxic responses in rats and hamsters. In pigs 500 mg 2,4-D acid/kg diet caused the sow to be anorexic and produced weakened piglets with 10 to 15 dying within one day after birth. When the five surviving piglets were fed the same diet for eight months they showed a marked depression in growht. Sheep have tolerated oral doses of 2,4-D acid for 30-90 days at 2 grams per day levels, while reindeer experienced no adverse effects from daily oral doses of 1 mg/kg for 30-54 days.

E. Carcinogenic and Tumorigenic Potentials of 2,4-D

Studies of the carcinogenic properties of 2,4-D in mammalian biological systems are limited at best. However, in an extensive study by Innes et al (67), the tumorigencity of some 130 test compounds were tested in mice. Included in the test compounds were the 2,4-D acid and the isopropyl, butyl and isooctyl esters of 2,4-D. They were given orally, at a daily dosage rate of 46.4 mg/kg. An additional test using the dosage rate of 100 mg/kg for 2,4-D acid was included. These doses were given by stomach tube starting at 7 days of age and continued until the mice were 4 weeks of age. After weaning, the test compounds were mixed directly into the diet and the same dosage rate maintained for approximately 18 months of observation. The tumor incidence in any group or combination of groups in which 2,4-D was tested was not significantly different from that in control animals.

Groups of male and female mice were given single subcutaneous injections of 215 mg/kg 2,4-D in dimethyl sulphoxide (DMSO) on the 28th day of life and observed up to 78 weeks of age. Tumor incidences in any group or combination of groups were not significantly different from that in controls. No increase in the incidence of tumors was observed in similar groups of mice treated with single subcutaneous injections of 21.5 mg/kg butyl or 100 mg/kg isopropyl esters of 2,4-D, both 99 percent pure. Mice treated with 21.5 mg/kg isooctyl ester of 2,4-D, 97 percent pure, had 5/17 females of one strain developing reticulum-cell sarcomas (12).

Walker et al (141) demonstrated that six, daily, intraperitoneal, injections of highly purified 2,4-D (99.0 percent) at the rate of 62 mg/kg effectively inhibited development of the Ehrlich ascites tumor maintained in BALB/c mice.

TABLE 3. Summary of literature data on the embryotoxic, fetotoxic and teratogenic potentials of 2,4-D in amimals

Animal	Number Used	Route of Administration	Response	Dose	Reference
Rat					
	PF[a] NS[b]	Drinking water during pregnancy.	No effect	1000 mg/l water[c]	15
	PF NS	Diet for 95 days then mated and continued through gestation and lactation.	Small birth wt. 94% died before weaning.	2000 mg/kg diet[d]	58
			Some reduction in birth wt. Some deaths in pups.	1000 mg/kg diet[d]	58
	25 F[e]	In diet for 2 yrs.	No effect	1250 mg/kg diet[d]	58
	25 M[f]	In diet for 2 yrs.	No effect	1250 mg/kg diet[d]	58
	PF NS	In diet 3 generations. Six litter reproduction study.	No effect	500 mg/kg diet[d]	58
			No effect on fertility of either sex nor litter size. Reduced % of pups born and surviving to weaning - lowered weaning weights.	1500 mg/kg diet[d]	58
	19 PF	Daily oral dose - days 6-15 of gestation.	No effect on fertility, gestation, lactation or viability of newborn.	87.5 mg/kg[g]	122
	119 Fetuses	Daily oral dose to females - days 6-15 of gestation.	Fetotoxic as edema, delayed ossification, wavy ribs. No teratogenicity.	87.5 mg/kg[g]	122

Table 3 continued

	PF NS	Daily oral dose - days 6-15 of gestation	Minimal fetopathy, increased skeletal anomalies	150 mg/kg[h]	74
Hamsters					
	PF NS	Daily oral dose - days 6-15 of gestation	No consistent embryotoxic effects	100 mg/kg[d]	24
Sheep					
	PF	Via stomach tube 30, 60 or 90 days	No effect	2 g/day[d]	10
Pig					
	1 PF	In diet throughout pregnancy.	Female anorexic. 10 of 15 piglets died in 24 hrs.	500 mg/kg diet[d]	15
	5 Newborn	In diet for 8 months	Growth depression, anemia, moderate liver and kidney lesions.	500 mg/kg diet[d]	15
Reindeer					
	15 PF	In diet for 1 - 1.5 months	No effect	1 mg/kg[d]	40

[a] PF - pregnant female

[b] NS - number of animals in study not stated or unavailable from literature source

[c] Sodium salt of 2,4-D

[d] 2,4-D acid

[e] F - Female

[f] M - Male

[g] 2,4-D acid or molar equivalents of propylene glycol butyl ether ester of 2,4-D or the isooctyl ester of 2,4-D

[h] 2,3-D acid or butyl or isooctyl esters of 2,4-D

Hansen et al (58) studied groups of 25 male and 25 female Osborne-Mendel rats that were fed for two years on diets containing 2,4-D at 0, 5, 25, 125, 625 or 1,250 mg/kg levels. The 2,4-D was 96.7 percent pure and contained no detectable levels of 2,7-dichloro or 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (limit of sensitivity of method of analysis was 1 mg/kg). No target organ tumors were observed and the individual tumor types were randomly and widely distributed and of the type normally found in aging rats of that strain. Statistical analysis of the randomly distributed tumor types indicated a tendency for the proportion of females with tumors to increase with 2,4-D dosage and a trend toward dose related increases in the proportion of males with malignant tumors. The number of treated rats with malignant tumors over controls was found only in males receiving the highest dosage level.

A review of the summary of the literature on the carcinogenic and tumorigenic potentials of 2,4-D in animals presented in Table 4, revealed that 2,4-D acid, and the isopropyl, butyl and isooctyl esters of 2,4-D did not adversely affect nor increase the incidence of tumors in test animals when fed at levels of 46.4 to 100 mg/kg of diet to mice or 1,250 mg/kg of diet to rats for 18 to 24 months. Those tumors that did occur were not necessarily in target organs and were the type tumors normally seen in aging laboratory animals of the species and strain being studied. Single subcutaneous injections of 21.5 to 215 mg/kg of 2,4-D acid, isopropyl and butyl esters of 2,4-D in DMSO did not produce carcinogenic or tumorigenic responses in male or female mice. A single subcutaneous injection of 21.5 mg/kg of the isooctyl ester of 2,4-D in DMSO did produce an increased incidence of reticulum-cell sarcomas in treated female mice. It should be noted that DMSO itself is now considered to be a potential carcinogen. At 62 mg/kg, 2,4-D acid injected intraperitoneally in mice inhibited the development of Ehrlich ascites tumor being maintained in mice.

F. Mutagenic and Cytogenetic Potentials of 2,4-D in Animals

Most of the mutagenic studies of 2,4-D have been conducted in bacterial cultures or in plant and animal tissue cultures; however, Styles (133) investigated the cytotoxic effects of 2,4-D on *in vivo* and *in vitro* test systems and found no increase in mutation rate and no evidence of mutagenicity in the test rats. He found serum from orally dosed rats was not mutagenic to *Salmonella typhimurium*. Complete details of this study were not readily available.

Pilinskaya (101) observed that treatment of cultured human lymphocytes with 2.5 X 10^{-7} M (0.02 μg/ml) 2,4-D increased the number of chromatid aberrations (single acentric fragments) and, to a lesser extent, the chromosomal aberrations (paired acentric fragments). In mice, Pilinskaya (101) found toxic concentrations (100-300 mg/kg) of 2,4-D administered as a single oral dose significantly increased the frequency of aberrant metaphases (2-4 fold) with single fragments being the aberration seen.

TABLE 4. Summary of literature data on the carcinogenic and tumorigenic potentials of 2,4-D in animals

Animal	Number Used	Route of Administration	Response	Dose	Reference
Mouse					
	18 M[a]/18 F[b] of two hybrid strains	Stomach tube, beginning at 7 days of age for 21 days, then in diet for 18 months	No effect	40.4 mg/kg diet[c]	67
			No effect	100 mg/kg diet[d]	67
	M - F NS[e]	Single subcutaneous injections in DMSO	No effect	215 mg/kg[d]	12
			No effect	21.5 mg/kg[f]	12
			No effect	100 mg/kg[g]	12
			5/17 females developed reticulum-cell sarcomas	21.5 mg/kg[h]	12
	8	6 daily intraperitoneal injections	Inhibited development of Erlich ascites tumor	62 mg/kg[d]	
Rats					
	25 M/25 F	Diet for 2 years	No target organ tumors, random type tumors normally seen in aging rats	1250 mg/kg diet[c]	58

[a] M - Male

[b] F - Female

[c] 2,4-D acid and isopropyl, butyl and isooctyl esters of 2,4-D

[d] 2,4-D acid

[e] NS - number of animals in study not stated or unavailable from literature source

[f] Butyl ester of 2,4-D

[g] Isopropyl ester of 2,4-D

[h] Isooctyl ester of 2,4-D

Jenssen and Renberg (68) found there was no detectable increase of micronuclei in the erythrocytes of mouse bone marrow after intraperitoneal administration of 100 mg/kg 2,4-D. Because of the high experimental resolution power of the test system used in this study it was particularly suitable for the detection of weak chromosome breaking activity of 2,4-D in mammals. The lack of penetration of 2,4-D into the cells was in accordance with the rapid excretion that is known to occur in the mammalian body. This experiment did not in the authors opinion, consititute a reliable measure of the mutagenic potential of 2,4-D; however, in practice the lack of penetration of this substance into the cells indicated it does not constitute a cytogenetic hazard to man.

Epstein et al (37) found that 2,4-D did not increase dominant lethal mutations in mice when given as a single intraperitoneal injection of 125 mg/kg or when given orally on five successive days for a total dose of 75 mg.

In host-mediated assays Zetterberg et al (146) using *Salmonella typhimurium* strains TA1530 and TA1531, or *Saccharomyces cerevisiae* D4, found no mutagenic effects in the organisms when host adult male mice were given 6 mg 2,4-D (200 mg/kg) by gavage.

Bongso and Basrur (17) exposed embryonic bovine kidney cells and bovine peripheral blood cells, *in vitro*, to concentrations of 1-1,000 µg/ml 2,4-D for 6-96 hours resulting in stimulation of mitosis. Chromosomal aberrations were not detected in the peripheral blood cells, but nucleolar irregularities and polyploid mitotic stages were observed in the kidney cells.

Andersen et al (4) evaluated 110 herbicides for their ability to induce point mutation in one or more of 4 different microbial systems. The herbicide 2,4-D was included in this study. The authors did not state the purity of the compounds being tested. The 2,4-D did not cause point mutations in these microbial systems in comparison with known mutagens such as 5-bromouracil or 2-aminopurine. These observations of no mutagenicity of 2,4-D in *Escherichia coli* WP2 her^+ or her^- or in *Salmonella typhimurium* strains TA1535, TA1536, TA1537 or TA1538 were also confirmed in works by Nagy et al (94), Shirasu (126) and Shirasu et al (127).

A review of the literature on the mutagenic and cytogenic potentials of 2,4-D in animals generally supported the premise that 2,4-D was not highly cytotoxic in laboratory animals. It did not increase mutation rates nor stimulate a mutagenic response in rats and mice. In various *in vitro* and *in vivo* test systems 2,4-D did cause chromatid aberrations and nucleolar irregularities in cultured human lymphocytes, bovine kidney cells and tissues of mice given single toxic doses. No mutagenic responses were seen in several studies using microbial systems for the detection of mutagenic and cytogenic responses to 2,4-D.

III. REVIEW OF 2,4,5-T TOXICITY IN ANIMALS

A. The Acute and Short-Term Toxicity Potentials of 2,4,5-T

Detailed accounts of the experimental procedures used to study the acute and short-term toxicity potentials of 2,4,5-T were not available. References to acute toxicity of 2,4,5-T in small laboratory animals referred to a summary article on toxicological information on 2,4-D and 2,4,5-T by Rowe and Hymas in 1954 (115). Their literature review made reference to the 1953 work of Drill and Hiratzka (33) where acute and chronic oral toxicity studies on 2,4-D and 2,4,5-T were conducted with dogs. Apparently, the earlier research with small laboratory animals dealt primarily with 2,4-D, although some 2,4,5-T studies conducted in 1950 discussed effects on horses, dairy and beef cattle, sheep, swine and chickens immediately pastured on freshly treated alfalfa. Unfortunately, Rowe and Hymas did not detail the methodology for obtaining all the data that appeared in their article. Table 5 presents the available data from the literature dealing with the acute toxicity of 2,4,5-T in laboratory animals.

Drill and Hiratzka (33) administered commercial 2,4,5-T of 98.9 percent purity (TCDD level not stated) in a single oral dose of 50, 100, 250 and 400 mg/kg to a total of 10 adult mongrel dogs of both sexes. The 400 and 250 mg/kg dosages were given to individual male dogs and both died in 2 and 3 days, respectively. One of four female animals died at the 100 mg/kg dose level; however, no other signs of toxicity were noted. All three males and one female in the 50 mg/kg dosage level survived with no signs of toxicity. The acute oral LD_{50} for 2,4,5-T acid was in the range of 100 mg/kg or higher for dogs. Toxic doses of this level produced only mild signs of muscle spasticity.

Björklund and Erne (15) fed single oral doses of 100 mg/kg 2,4,5-T to pigs causing anorexia, vomiting, diarrhea and ataxia. At autopsy, hemorrhagic enteritis and congestion of the liver and kidney were found [see IARC Monograph, Vol 15 (66)].

Study of the data in Table 5 indicated that the various forms of 2,4,5-T all fall in the same range of acute toxicity for mice, rats, guinea pigs and rabbits. The dog appeared to be somewhat more susceptible. The LD_{50} values for 2,4,5-T and its common derivatives were in the range of 380 to 940 mg/kg for the small laboratory animals. When given orally to dogs, even in fatal cases, 2,4,5-T produced only weak signs in the form of ataxia and stiff movements of the hindlegs.

B. The Subacute and Chronic Toxicity Potentials of 2,4,5-T

Highman et al (62) conducted a study using 978 mice including control animals. On days corresponding to days 6 through 14 of pregnancy, groups of pregnant and nonpregnant CD-1 mice and male and nonpregnant

TABLE 5 . Summary of literature data on the no-effect LD_{50} and LD_{100} levels of the acute toxicity of 2,4,5-T in animals

Animal	Number Used	Route of Administration	Dose-Toxicity	Single Dose mg/kg	Reference
Mouse					
	NS[a] M[b]	Oral in olive oil	LD_{50}	389[c]	115
	NS F[d]	Oral in olive oil	LD_{50}	551[e]	115
	NS F	Oral in corn oil	LD_{50}	940[f]	115
Rat					
	NS M	Oral in olive oil	LD_{50}	500[c]	115
	NS M & F	Oral in olive oil	LD_{50}	495[e]	115
	NS F	Oral in corn oil	LD_{50}	481[f]	115
	NS F	Oral in olive oil	LD_{50}	750[g]	115
Guinea Pig	NS M & F	Oral in olive oil	LD_{50}	381[c]	115
	NS F	Oral in olive oil	LD_{50}	449[e]	115
	NS F	Oral in corn oil	LD_{50}	750[f]	115
Rabbit					
	NS M	Oral in corn oil	LD_{50}	712[f]	115

Table 5 continued

Dog	1 M	Oral in capsule	LD_{100}	250[c]	33
	4 F	Oral in capsule	LD_{50}[h]	100[c]	33
	1 F 3 M	Oral in capsule	No effect	50[c]	33
Pig	NS	Oral	Anorexia, vomiting, diarrhea, ataxia, hemorrhagic enteritis, liver and kidney congestion.	100[c]	15

[a] NS - number of animals in study not stated or unavailable from literature source.

[b] M - Male

[c] 2,4,5-T acid

[d] F - Female

[e] Isopropyl ester of 2,4,5-T

[f] Mixed butyl esters of 2,4,5-T

[g] Mixed amyl esters of 2,4,5-T

[h] One of four animals died on 7th day

female dihybrid cross F_2 mice received, by gavage, 2,4,5-T acid doses ranging from 30 to 140 mg/kg. Some groups received a technical preparation of 2,4,5-T (97.9 percent pure, containing < 0.05 mg/kg TCDD or a purified preparation of 2,4,5-T (99 percent pure, containing < 0.05 mg/kg TCDD). Mice killed when they became moribund and at 1, 2, 4, 6, 8 and 11 days after beginning treatment. Sick or moribund mice sacrificed after 2-9 doses of 2,4,5-T often showed severe myocardial lesions, hypocellularity of the bone marrow and depletion of lymphocytes in the thymus, spleen, or lymph nodes. They also showed marked hematologic and blood chemistry changes. Treated mice remaining healthy showed few or no lesions and no blood chemistry changes, but often developed a mild anemia attributable to a hemolytic effect of 2,4,5-T. The incidence of animals becoming moribund was less than 1 percent in the CD-1 mice, including those given 140 mg/kg, and ranged from 53 to 82 percent in groups of male and female F_2 mice receiving 120 mg/kg 2,4,5-T. The incidence of moribund mice tended to be higher in male than in female F_2 mice and in those given the purified compound. The findings of this study indicated that impairment of maternal health by severe lesions early in gestation were not the primary cause of an increased incidence of fetal abnormalities observed in mice given 2,4,5-T. The lesions appeared to be due primarily to 2,4,5-T, rather than to contaminants in the technical preparation. Finally, the importance of using more than one strain of mouse in toxicological studies was vividly illustrated.

Highman et al (60) using 378 pregnant dihybrid cross F_2 female mice gave either 60 or 120 mg/kg 2,4,5-T via gavage on days 6 through 14 of gestation. Both technical (97.9 percent pure with less than 0.005 mg/kg TCDD) and a more purified preparation (99 percent pure with less than 0.005 mg/kg TCDD) of 2,4,5-T were used in this study. Mice were killed when they became moribund and at 6, 24, and 30 hours, as well as at 4, 6, 8 and 11 days after beginning treatment. Mice given 60 mg/kg and many given 120 mg/kg 2,4,5-T appeared normal at the time they were terminated either in early or late pregnancy and showed few or no pathologic changes. Mice that became ill or moribund often showed severe lesions and few survived past 11 days. The histopathological lesions included myocardial rarefaction and necrosis, thymus cortical atrophy, splenic atrophy and hypocellularity in bone marrow and lymph nodes.

Groups of 10 male and 10 female rats per test dose were fed for 90 days on diets containing 2,4,5-T at the daily dosage levels of 0, 3, 10, 30 or 100 mg/kg body weight. The 2,4,5-T acid was from commercial production and contained less than 1 mg/kg TCDD. No effects were noted in the animals fed 3, 10 or 30 mg/kg doses. Changes found in both sexes fed 100 mg/kg included depression in body weight gain, slight decrease in food intake and elevated serum alkaline phosphatase levels. Male rats at this dose had slightly increased serum glutamic-pyruvic transaminase levels and slight decreases in red cell counts and hemoglobin. Inconsistent hepatocellular swelling was observed upon histopathological examination in some livers. [See World Health Organization (WHO) Monograph 71.42 (143), and IARC Monograph, Vol 15 (66)].

Groups of 10 male and 10 female rats per test dose were fed for 90 days on diets containing 0, 100, 300, 1,000 and 3,000 mg/kg of food, of the mono-, di-, and tripropylene glycol butyl ether esters of 2,4,5-T. The 2,4,5-T acid equivalent was 62 percent. No effect levels were 100 and 300 mg/kg of diet. At 1,000 mg/kg level slight cloudy swelling of the parenchymal cells with central lobular necrosis was noted in two of ten animals examined. Kidney weights were increased with mild cellular changes noted such as cloudy swelling of renal tubular epithelium. At 3000 mg/kg a significant retardation in growth was noted in males but not females, liver and kidney weight increased in males, livers were large and light in color in both sexes, generalized cloudy swelling of liver cells and slight central lobular necrosis and cloudy swelling of renal tubular epithelium [see WHO Monograph 71.42 (143) and IARC Monograph, Vol 15 (66)].

Konstantinova (81) conducted experiments with pregnant rats using 12-13 animals per treatment group and dosing them via stomach tube for the entire gestation period with 0.01, 0.1, 0.42 and 4.2 mg 2,4,5-T/kg body weight. The butyl ester of 2,4,5-T was used in this study; however, source and purity were not stated in the translation. No effects were noted at the 0.01 mg/kg dose. The threshold level in this study was at the 0.1 mg/kg dose. At 0.42 mg/kg a general toxic effect on pregnant females was noted and embryotoxic effects were of an irregular character and difficult to evaluate. The 4.2 mg/kg produced a significant increase in total embryo fatalities, a decrease in the number of live offspring (average one less per female) and a decrease in the weight of the offspring.

Rip and Cherry (111) fed a group of 12, four-week-old, male, Long-Evans rats, analytical standard grade 2,4,5-T (containing no detectable TCDD at a sensitivity of 0.05 mg/kg) mixed with the diet at a rate of 10 mg/animal/day for 1-11 days. Feeding of the 2,4,5-T caused liver enlargement with no effect on the weight of the kidneys, spleen, or body weight of the animal. Increases in relative liver weight were dose dependent and were observed after the first or second feeding. Enlargement was associated with substantial increases in total RNA and total protein per liver. The increases were not restricted to any particular subcellular fraction, but appeared to represent a general induction of RNA and protein synthesis. Total DNA content per liver was not affected. The enlargement response was reversible on the removal of the 2,4,5-T from the diet. This increase did not appear to be directed toward the synthesis of 2,4,5-T metabolizing enzymes. 2,4,5-T did not stimulate production of enzymes known to be produced by hepatotoxic compounds. This suggested that 2,4,5-T did not have a strong hepatotoxic activity and in fact it demonstrated activity similar to a structurally related compound chlorophenoxyisobutyrate (CPIB) which, like 2,4,5-T, induced liver enlargement and stimulated RNA and protein synthesis while inducing a strong self-metabolizing influence in the liver.

Drill and Hiratzka (33) administered 2,4,5-T acid via capsule to adult mongrel dogs, five days a week over a 13 week period. There was 1 male and 1 female in the 0, 2 and 5 mg/kg groups, a male and 2 females in the 10 mg/kg group and 2 males and 2 females in the 20 mg/kg group. All dogs in the 0, 2, 5 and 10 mg/kg dosage levels survived the 90-day test period. At 20 mg/kg the dogs died between days 11 and 75. The no effect level was 10 mg/kg per day. The histopathological examination of the 20 mg/kg dosed dogs was not remarkable and did not reveal a morphological cause of death. The prominent effects were weakness, slight stiffness in the hind legs, difficulty in swallowing food and in one dog, bleeding from the gums.

Study of the data in Table 6 indicated that the various forms of 2,4,5-T fell in the same general range of chronic toxicity for mice, rats and sheep. The exception to this statement were data presented by Konstantinova (81) using the butyl ester of 2,4,5-T of unstated purity. In mice there appeared to be a definite strain difference in susceptibility to 2,4,5-T toxicity. The no effect level in mice ranged from 30-120 mg/kg with an overlapping of adverse effects from 60-140 mg/kg in various strains of mice. Rats appeared to be tolerant to about 10 times the 2,4,5-T calculated dose when administered as mg/kg of diet as opposed to mg/kg body weight of the animal. The no effect level for rats fed 2,4,5-T chronically were approximately 30 mg/kg body weight and 300 mg/kg diet. Threshold toxicity levels for rats were approximately 100 mg/kg body weight and 1,000 mg/kg diet.

C. Absorption, Distribution and Exretion of 2,4,5-T

Single subcutaneous administration of 100 mg/kg 2,4,5-T to mice resulted in 23 percent of the dose being recovered in the body over a 24-hour period (147). In rats, 85.8 percent of a single intravenous dose of 100 mg/kg was found in the urine within 6 days (117).

Single oral doses to rats of 100 mg/kg of the triethanolamine salt of 2,4,5-T were readily absorbed, distributed and eliminated; excretion was primarily via the kidneys (38). Seven days after oral administration of 50 mg/kg 2,4,5-T (99.6 percent pure) to rats, 56-69 percent of the dose was recovered in the urine; 70-85 percent of the recovered dose was unchanged 2,4,5-T, and approximately 15-30 percent was found as the glycine and taurine conjugates and as 2,4,5-trichlorophenol; the two conjugates were excreted in nearly equal amounts (16, 55). Similar results were obtained in mice, except that the quantity of the taurine conjugate was greater (54).

The biological half-life of 5 mg/kg 2,4,5-T administered orally to dogs (77 h) was longer than that in rats (4.7 h). When the dose of 2,4,5-T to rats was increased to 200 mg/kg the biological half-life was prolonged to 25 h, indicating that the excretory capacity of the animals could be exceeded (104).

TABLE 6. Summary of literature data on the subacute and chronic toxicity of 2,4,5-T in animals

Animal	Number Used	Route of Administration	Effect	Dose	Reference
Mouse					
	978; F[a] PF[b] M[c]	Gavage, dosed daily for 6-14 days, corn oil vehicle	Varied by strain	30-140 mg/kg[d]	62
	CD-1 strain		<1% moribund	140 mg/kg[d]	62
	F_2 dihydrid		53-82% moribund	120 mg/kg[d]	62
	NCTR strain		Ill or moribund	60 mg/kg[d]	62
	CRBL strain		No effect	90-120 mg/kg[d]	62
	378; PF	Gavage, dosed daily for 6-14 days, corn oil vehicle	Varied	60-120 mg/kg[d]	60
			Most all animals outwardly[e] normal	60 mg/kg[d]	60
			Many animals outwardly[e] normal	120 mg/kg[d]	60
Rat					
	10 M/10 F	Daily dose per animal in feed for 90 days	No effect	30 mg/kg[f]	66
	10 M/10 F	Daily dose per animal in feed for 90 days	Anorexia, depressed weight gain	100 mg/kg[f]	66
	10 M/10 F	90 days in diet	No effect	300 mg/kg[g]	66

Table 6 continued

	10 M/10 F	90 days in diet	Toxicity; no deaths due to treatment; histopath changes were noted	1000 mg/kg diet^{g}	66
	10 M/10 F	90 days in diet	Growth retardation in males not females; no deaths due to treatment; histopath changes were noted	3000 mg/kg diet^{g}	66
	12 or 13/PF	Stomach tube, daily dosing, entire gestation.	No effect	0.01 mg/kg^{h}	81
	12 or 13/PF	Stomach tube, daily dosing, entire gestation.	Threshold level	0.1 mg/kg^{h}	81
	12 or 13/PF	Stomach tube, daily dosing, entire gestation.	Irregular effect on dam and fetuses	0.42 mg/kg^{h}	81
	12 or 13/PF	Stomach tube, daily dosing, entire gestation.	One less live pup per female, toxic signs noted.	4.2 mg/kg^{h}	81
	12 M	In diet to provide daily dose indicated	Liver enlargement only	10 mg/kg^{i}	111
Dog					
	1 M/1 F	Via capsule for 90 days	No effect	10 mg/kg^{j}	33
Sheep	2 M/2 F	Via capsule for 90 days	All died	20 mg/kg^{j}	33
	NS^{k}	Oral for 35 days	No effect	100 mg/kg^{l}	29^{m}

^{a}F - Female

^{b}PF - Pregnant female

^{c}M - Male

Table 6 continued

[d] Both technical and purified 2,4,5-T acid containing <0.05 and 0.005 mg TCDD/kg.

[e] Histopathological information presented in text.

[f] 2,4,5-T acid from commercial production containing <1 mg TCDD/kg.

[g] Mono-, di-, and tripropylene glycol butyl ether esters of 2,4,5-T, 62% 2,4,5-T acid equivalent.

[h] Butyl ester of 2,4,5-T, purity not stated.

[i] Analytical standard grade 2,4,5-T acid containing <0.05 mg TCDD/kg.

[j] Commercial 2,4,5-T acid, 98.9% purity, TCDD level not stated.

[k] NS - number of animals in study not stated or unavailable from literature source.

[l] Form not known.

[m] From table in reference (29).

Similar results were obtained with single intravenous injections of 5 or 100 mg/kg ^{14}C-2,4,5-T in rats, where 2.3 and 7.6 percent of the radioactivity were excreted in the feces, respectively, suggesting that at the higher dose biliary excretion of 2,4,5-T and/or its degradation products was involved in the overall elimination of 2,4,5-T from the body (117).

Marked differences in the pharmacokinetics of 2,4,5-T were seen with different species, ages and doses: clearance of 2,4,5-T from the plasma and body of dogs, mice and man was slower than that in rats. The volume of distribution after a single oral dose of 5 mg/kg also differed: in man, 0.079; in rats, 0.14; and in dogs, 0.22 l/kg (49, 104).

A single dose of 100 mg/kg 2,4,5-T to pregnant mice was almost entirely eliminated in 72 h; however, after 4 daily administrations of the same dose, 2,4,5-T accumulated in maternal tissues and fetuses, and by 48 h 2,4,5-T was still detectable throughout the fetuses (34).

No radioactivity was found in NMRI strain mouse embryos in an early stage of gestation after administration of ^{14}C-2,4,5-T to the dams. When given in late gestation, the fetal tissue had a level similar to that in maternal tissue (83). Selective uptake of 2,4,5-T into the yolk sac epithelium and absence of placental transfer in early pregnancy were effects similar to those seen with trypan blue in mouse embryos (84).

No radioactivity was detected in hamster embryos in a late stage of gestation after similar administration of 2,4,5-T to the dams (31).

The biological half-life of ^{14}C-2,4,5-T was significantly longer in newborn than in adult rats (41, 64). Radioactivity was found in all tissues examined as well as in milk and fetuses after a single oral administration of 0.17-41 mg/kg ^{14}C-2,4,5-T to pregnant rats (41).

Care must be taken when making all inclusive or generalized statements on the absorption, distribution and excretion of 2,4,5-T due to the demonstrated and marked differences in the pharmacokinetics of 2,4,5-T seen in laboratory animals. Such variables as age, dosage levels, routes of administration and chemical formulations all contributed to variations in response. Single doses of 2,4,5-T appeared to be rather quickly eliminated, primarily unchanged, via the urine and feces in a few hours up to about 7 days. High doses and repeated lower doses of 2,4-D or 2,4,5-T accumulated in animal tissues. The liver appeared to take a more active role in the metabolism and excretion of higher or chronic doses of 2,4,5-T than when single lower level doses were administered.

D. Embryotoxic, Fetotoxic and Teratogenic Potentials of 2,4,5-T

When reviewing the literature dealing with the embryotoxic, fetotoxic and teratogenic potentials of 2,4,5-T, care must be taken to note the levels of TCDD contamination that may have been present in the 2,4,5-T tested. The TCDD contamination may very well have ranged from undetectable levels, using analytical technology available at the time, to 30 ppm or more. In an extensive 1977 review article of the teratogenic effects of environmental chemicals, Wilson (145) stated that 2,4,5-T had been intensively examined in pregnant animals of six different species. A low level of teratogenicity had been demonstrated in three rodent species: rats, mice and hamsters. Tests in pregnant rabbits, sheep and rhesus monkeys have been negative. Wilson discussed these studies in detail in the test, *Handbook of Teratology* (144).

Doses of more than 30 mg/kg 2,4,5-T (containing <0.1 mg/kg TCDD) increased the frequency of cleft palates in some strains of mice. When similar doses were administered to pregnant mice on days 6-15 of gestation some fetal growth retardation was observed (13).

Courtney et al (26) first reported that under laboratory conditions 2,4,5-T was implicated as being teratogenic and fetotoxic. The 2,4,5-T used in the study was later found to contain 30 mg/kg TCDD. The 2,4,5-T was administered either orally or subcutaneously at a dose rate of 113 mg/kg per day on days 6-14 of gestation in C57Bl/6 mice and days 6-15 in AKR mice. Oral administration caused an increased incidence of cleft palate and fetal mortality in both strains and cystic kidneys in the C57Bl/6 mice. Subcutaneous injection resulted in significant increases in the incidence of cleft palate and cystic kidneys in the embryos of both strains of mice and evidence of fetal mortality in the C57Bl/6 mice.

Roll (112) found embryotoxic and teratogenic effects in NMRI mice exposed to 2,4,5-T, containing 0.05 ppm TCDD, administered orally at 20 to 130 mg/kg daily from 6 to 15 days of gestation. At 90 or 130 mg/kg/day the percentages of resorptions and/or dead fetuses were markedly increased relative to the controls. These levels also produced maternal toxic effects. Dose related reductions in fetal weight were observed at levels of 20 mg/kg/day and above. Cleft palate increased among fetuses exposed to 35 mg/kg/day or more. The teratogenic no effect level in mice for this particular sample of 2,4,5-T was considered to be 20 mg/kg/day. This was later confirmed with specially prepared samples of 2,4,5-T with no detectable (<0.02 mg/kg) amount of TCDD (112, 113).

Neubert and Dillmann (96) found that samples of 2,4,5-T acid containing less than 0.02 mg/kg TCDD produced embryotoxic effects in NMRI mice in the form of fetal weight reductions at levels as low as 10 and 15 mg/kg per day, given orally from day 6 to 15 of gestation. The butyl ester of 2,4,5-T showed similar embryotoxic effects in mice when administered in the same manner. Cleft palates were produced using single doses of 2,4,5-T acid at 150-300 mg/kg. The maximum teratogenic effect was seen when mice were dosed on day 12 or 13 of gestation. A potentiation of the teratogenic effects (cleft palate) of 2,4,5-T and TCDD was obtained when teratogenic doses of one of the substances was combined with threshold doses of the other. However, for clear-cut potentiation of the effect of 30-60 mg/kg 2,4,5-T acid more than 1.5 mg/kg TCDD was required. When the level of TCDD drops below 1 mg/kg it was predicted that there would be no additional contribution to the embryotoxic effects of 2,4,5-T (i.e., cleft palate) in NMRI mice. It was concluded that in some of the 2,4,5-T preparations there must have been other contaminants present which exaggerated the teratogenic effect to some extent. Such contaminants may have been present in more than trace amounts. For example, 2,4,5-trichlorophenol, did not contribute significantly to the teratogenic effect.

The effect of 2,4,5-T and TCDD were studied in random bred CD-1 and inbred DBA/2J and C57Bl/6 strains of mice by Courtney and Moore (27). Two different samples of 2,4,5-T and one sample of TCDD were used. The 2,4,5-T technical grade contained 0.5 mg/kg TCDD and the analytical grade contained less than 0.05 mg/kg TCDD. Compounds were administered subcutaneously from day 6 to day 15 of pregnancy as solutions in 100 percent dimethyl sulfoxide (DMSO) in a volume of 100 µl/animal/injection. Both samples of 2,4,5-T and TCDD produced cleft palate in all three strains of mice when 2,4,5-T was administered at levels of 100 mg/kg and TCDD at 3 µg/kg. Kidney malformations were produced by both 2,4,5-T samples in CD-1 mice and TCDD produced marked kidney anomalies in all mice strains. When 100 mg/kg 2,4,5-T and 1 µg/kg TCDD were administered in combination to CD-1 mice, the activity was not potentiated at the dose levels employed.

Bage et al (5) injected NMRI mice subcutaneously with 50 and 110 mg/kg 2,4,5-T containing less than 1.0 ppm dioxin on each of days 6 through 14 of gestation. At 110 mg/kg 2,4,5-T was teratogenic, causing cleft palates, rib and vertebrae anomalies as well as being fetotoxic causing 25 to 35 percent resorptions.

Highman et al (61) recently reported that it was possible to detect a retardation in renal alkaline phosphatase in fetal kidneys from fetuses of mice given doses of 2,4,5-T by gavage at the rate of 60-120 mg/kg on days 6-14 of pregnancy. This retardation in renal alkaline phosphatase levels was suggested as the cause for the delay in renal functional development and indirectly supported the view that 2,4,5-T caused retarded development, rather than true teratogenesis. In this study a reduction of fetal weight and an increase in the incidence of cleft palate were seen in fetuses from treated females.

Frohberg et al (45) administered 0, 20, 40, 80 and 120 mg/kg 2,4,5-T acid or butoxyethyl ester containing <0.1 mg/kg dioxin, by the oral route to NMRI mice. Oral doses of 80-120 mg/kg 2,4,5-T acid or 120 mg/kg butoxyethyl ester were required to produce malformations and fetal deaths. In the inhalation experiments, 216 mg/m^3 of the butoxyethyl ester showed a slight maternal toxic and fetotoxic and teratogenic effect. Ten exposures to 374 mg/m^3 killed 5 of 15 dams, while 392 mg/m^3 from day 11-15 of gestation was toxic for the dam and caused fetal deaths.

Sparschu et al (130) orally administered commercial grade 2,4,5-T containing 0.5 mg/kg TCDD, to rats in daily doses of 50 and 100 mg/kg on days 6 to 15 and 6 to 10 of pregnancy, respectively. At the lower dosage level minimal fetal effects were seen with a slightly higher incidence of delayed ossification of the skull bones being observed. The higher level was toxic to the dams and caused a high incidence of maternal deaths. Only 4 of 25 rats survived with three showing complete, early, fetal resorptions and one had a litter of 13 viable fetuses which showed toxic effects but no evidence of teratogenic anomalies.

Khera and McKinley (74) found that 2,4,5-T, containing less than 0.5 mg/kg TCDD, induced some fetopathy and increased the incidence of skeletal anomalies in Wistar rats following single daily oral doses of 100-150 mg/kg on days 6-15 of gestation. The butyl ester produced no grossly observable teratologic effects when given at doses of 50 or 150 mg/kg. Various formulations of 2,4,5-T given to pregnant female rats demonstrated that a teratologic potential existed, in the form of skeletal anomalies, when repeated doses of 100 mg/kg or greater were given. At 25 mg/kg 2,4,5-T negative results were noted while at 50 mg/kg effects were noted but were not significant (P=0.05) when compared to control animals. The butyl ester produced no grossly observable anomalies and no adverse effects on the postnatal survival when pregnant females were treated at 50 and 150 mg/kg. Three of 8 females died at the 150 mg/kg dose. Skeletal deformities noted were not incompatible with life and no adverse change in reproductive performance or behavioral characteristics were detected. In the authors opinion, the predictive value of postnatal studies in relation to the detection of the teratogenic potential of test compounds may not be reliable on its own.

Courtney et al (26) found that when 4.6, 10 or 46.4 mg/kg/day of 2,4,5-T was given orally on days 10-15 of gestation to Sprague-Dawley rats, kidney anomalies and other embryotoxic signs were seen at all levels. Some rat fetuses were reported to have had hemorrhagic gastrointestinal tracts. At the highest level there was a 60 percent fetal mortality and a higher incidence of abnormalities in the survivors. Courtney and Moore (27) reported that in CD rats, 2,4,5-T orally administered at 10, 21.5, 46.4 and 80.0 mg/kg was neither teratogenic nor fetotoxic. Prenatal administration of 2,4,5-T did not effect the postnatal growth and development of the CD rat.

Sokolik (129) orally administered 2,4,5-T acid at dosage levels of 100 and 400 mg/kg per day and the butyl ester of 2,4,5-T at dosage levels of 50 and 200 mg/kg per day to rats on days 1 to 14 or 1 to 16. The purity of the 2,4,5-T in either form was not given. At 100 mg/kg

2,4,5-T produced embryos with a combination of deformities including absence of the lower jaw, changes in the hind limbs and exophthalmos. At the level of 400 mg/kg one embryo was found with tridactyly of the upper limb combined with syndactyl, while another embryo had brachydactylia of the upper limb. Both levels of the butyl ester of 2,4,5-T were more toxic than 2,4,5-T acid, causing 30 percent embryonic mortality at 200 mg/kg. The lower dose of 50 mg/kg caused high mortality among the embryos as well. At the higher level the butyl ester induced cleft palate, hydronephrosis, hydrocephalus and extensive gastrointestinal hemorrhages along with hind limb brachydactylia. Cleft palate was the primary anomaly seen at the 50 mg/kg dosage level. Sokolik (129) concluded that the identical teratogenic action of the two preparations was probably attributable to the presence of dioxin, while the quantitative differences between the effects were attributable to differences in the concentration of the dioxin.

Konstantinova (81) conducted experiments in white rats where 2,4,5-T butyl ester, purity not stated, was given orally to pregnant females for the entire period of the pregnancy at 0.01, 0.1, 0.42 and 4.2 mg/kg. The lowest level found to cause no effect was 0.01 mg/kg in the water. The threshold level was considered to be 0.1 mg/kg, with 0.42 mg/kg showing a general toxic effect on the pregnant female rat; however, the changes noted in the embryos had an irregular character. The highest dose level, 4.2 mg/kg, had a general toxic effect causing nervous system dysfunction in the female rats, changes in peripheral blood and a relative increase in the weight of internal organs. The embryotoxic effects were increased embryo deaths, lowered offspring weight, hydrocephaly and peritoneal cavity hemorrhages.

In FW49 rats given daily oral doses of 25 to 150 mg/kg of either the TCDD-free or commercial grade 2,4,5-T (<0.1 ppm TCDD) showed no evidence of teratogenic effects (113).

Emerson et al (36) confirmed the lack of teratogenic and fetotoxic effects of 2,4,5-T when containing only 0.5 ppm TCDD and when given in daily doses, by gavage, at the levels of 1, 3, 6, 12 or 24 mg/kg in Sprague-Dawley rats. Moreover, doses up to 24 mg/kg 2,4,5-T containing 1 mg/kg TCDD had no teratogenic effect in rats when given on days 6-15 of gestation.

King et al (76) found no cleft palates when 93 embryos of Sprague-Dawley rats were injected *in utero* with purified 2,4,5-T on any one day ranging from 12 to 16 days of gestation at dosages of 50 to 125 μg/embryo. Two cleft palates were produced when technical grade 2,4,5-T was injected on day 15 of gestation into 118 embryos using the same techniques. In the control rats 45 females delivered 442 normal fetuses, with a 3.5 percent resorption rate and average liter size of 9.8

Commercial samples of 2,4,5-T containing TCDD in concentrations of 0.1, 0.5, 2.9 or 45 mg/kg caused fetal death and teratogenic effects in Syrian golden hamsters when given orally on days 6 through 10 of pregnancy at dosage levels of 20, 40, 80 or 100 mg/kg. As the dosage of 2,4,5-T increased and the TCDD content elevated, the effects were also increased. Pure 2,4,5-T containing no detectable TCDD produced no malformations when the dosage level was less than 100 mg/kg. Absence of eyelids (bulging eyes) and delayed ossification of the skull and exencephaly accounted for the main teratological abnormalities caused by 2,4,5-T containing TCDD. Hemorrhagic gastrointestinal tracts in the hamster fetuses appeared to be directly related to 2,4,5-T administration and could not be clearly linked to dose level of the compound or the dioxin content. These hemorrhages along with a marked edema noted in some of the fetuses reflected a toxic effect on fetal organs as opposed to a teratological effect (24).

Gale and Ferm (47) gave pregnant golden hamsters intravenous doses of 2,4,5-T on day 8 of gestation at the level of 2 mg/kg and found a 9 percent resorption rate in test animals compared to a 6 percent resorption rate in control animals. No malformed embryos were detected in this study; however, the purity of the 2,4,5-T was not given.

New Zealand rabbits given oral doses of 0, 10, 20 or 40 mg/kg 2,4,5-T (containing <0.5 mg/kg TCDD) on days 6-18 of pregnancy showed no evidence of embryotoxic or teratogenic effects in their offspring (36).

Dougherty et al (32) found that technical grade 2,4,5-T, containing 0.05 mg/kg TCDD was not teratogenic in rhesus monkeys, *Macaca mulatta*, when given at 0, 0.05, 1.0 or 10 mg/kg, nor did it interfere with normal development of the young. Groups of 10 pregnant females were treated daily with stomach tube from days 22-38 of pregnancy. There was no evidence of toxicity to the females at these levels.

In the 1971 Report of the Advisory Committee on 2,4,5-T (2), a preliminary study was cited where pregnant rhesus monkeys were orally dosed with 2,4,5-T, containing 0.05 mg/kg TCDD, at levels of 5, 10, 20 and 40 mg/kg three times weekly for 4 weeks between days 20-48 of pregnancy. After 100 days of gestation 12 fetuses were removed by hysterectomy and examined. All were found to be developmentally normal and their weight range was not significantly different than the control animals of the same age.

Binns and Balls (11) found no congenital deformities in lambs from ewes daily fed 100 mg/kg 2,4,5-T acid or the propylene glycol butyl ester of 2,4,5-T from the 14th to the 36th day of gestation. A third group of ewes fed 113 mg/kg of 2,4,5-T also showed no congenital deformities when fed at different periods during gestation.

A summary of the literature on the embryotoxic, fetotoxic and teratogenic potentials of 2,4,5-T in animals is presented in Table 7. In reviewing the literature it was evident that embryotoxic and teratogenic responses occurred in some strains of mice, rats and hamsters when repeated oral doses of 20 to 400 mg/kg 2,4,5-T was administered. Embryotoxicity and teratogenic studies in pregnant rabbits, sheep and rhesus monkeys have been negative. The embryotoxic and teratogenic potentials

TABLE 7 Summary of literature data on the embryotoxic, fetotoxic and teratogenic potentials of 2,4,5-T in animals

Animal	Number Used	Route of Administration	Response	Dose	Reference
Mouse	NS[a] PF[b]	Daily oral dose, days 6-15 of gestation	Increased frequency of cleft palate in some strains. Fetal growth retardation.	>20 mg/kg[c]	13
	NS PF C57Bl/6 strain AKR strain	Daily oral or s.c.[d] dose, days 6-14 of gestation	Oral increased cleft palate and fetal mortality, both strains.	113 mg/kg[e]	26
			Cystic kidneys in C57Bl/6		
			s.c. increased incidence of cleft palate and cystic kidneys in both strains.		
			Increase in fetal mortality in C57Bl/6 strain.		
	NS PF	Daily oral dose, days 6-15 of gestation	No effect	20 mg/kg[f]	112
			Cleft palate	35 mg/kg[g]	112
			Marked increase in resorptions and "4" dead fetuses.	90-130 mg/kg[g]	112

Table 7 continued

NS PF	Daily oral dose, day 6-15 of gestation	Fetal weight reduction	10-15 mg/kg^{f,h}	96
	Single dose during mid-gestation	Cleft palate, maximum teratogenic effect day 12 or 13 of gestation	150-300 mg/kg^{f}	96
	Daily oral dose, days 6-15 of gestation	Cleft palate	30-60 mg/kg^{i}	96
NS PF CD-1 strain DBA/2J strain C57Bl/6 strain	s.c. days 6-15 of gestation in a solution of DMSO at 100 µl/animal/injection	Cleft palate, all three strains	100 mg/kg^{j}	96
		Kidney malformations in CD-1 strain		
NS PF	s.c. days 6-14 of gestation	No effect	50 mg/kg^{k}	5
		Teratogenic, cleft palate, rib and vertebrae anomalies, fetotoxic	110 mg/kg^{k}	5
NS PF	Gavage, daily, days 6-14 of gestation	Retardation in renal alkaline phosphatase in fetal kidneys, no true teratogenesis, reduced fetal weight, cleft palate	60-120 mg/kg^{l}	61
NS PF	Daily oral dose, days 6-15 of gestation	Toxic to females, malformations and fetal death	80-120 mg/kg^{m} 120 mg/kg^{l}	45
	Inhalation of aerosol for 10 exposures	Slight maternal toxicity, fetotoxic, teratogenic	216 mg/m^{3,n}	45

Table 7 continued

		Inhalation of aerosol for 10 exposures	5-15 females died	374 mg/m^3[n]	45
		Inhalation of aerosol for 5 exposures	Toxic to females, fetal deaths	392 mg/m^3[n]	45
Rat	25, PF per test group	Daily oral dose,days 6-15 of gestation	Minimal fetal effects	50 mg/kg[o]	130
		Daily oral dose, days 6-10 of gestation	Toxic to females, high maternal death, 4 of 25 survived, 3 had complete fetal resorptions, 1 had a litter of 13 live fetuses, toxic but no anomalies	100 mg/kg[o]	130
	NS PF Wistar strain	Daily oral dose,days 6-15 of gestation	Fetopathy and skeletal anomalies	100-150 mg/kg[p]	74
		Daily oral dose, days 6-15 of gestation	No effect at 50 and 100 mg/kg. 150 mg/kg killed 3 of 8 females.	50-150 mg/kg[q]	74
	NS PF Sprague-Dawley strain	Daily oral dose, days 10-15 of gestation	Kidney anomalies, embryotoxic	4.6, 10 or 46.4 mg/kg[e]	26
			At 46.4 mg/kg, 60% fetal mortality, many abnormalities in survivors		
	NS PF	Daily oral dose, days 1-14 of gestation	Many deformities	100 mg/kg[r]	129
			Many limb abnormalities	400 mg/kg[r]	129

Table 7 continued

			Daily oral dose, days 1-16 of gestation	Embryo mortality, cleft palate	50 mg/kg^{s}	129
				30% embryo mortality and many anomalies	200 mg/kg^{s}	129
	NS	PF	Daily oral dose throughout pregnancy	No effect	0.01 mg/kg^{s}	81
				Threshold level	0.1 mg/kg^{s}	81
				Toxic to female, irregular embryotoxic effect	0.42 mg/kg^{s}	81
				Toxic to female, nervous signs, embryo deaths	4.2 mg/kg^{s}	81
	NS	PF	Daily oral dose during pregnancy	No effect	25-150 mg/kg^{c,t}	113
				No effect	1-24 mg/kg^{o}	36
	NS Sprague-Dawley strain	PF	Gavage, daily during pregnancy	No effect	24 mg/kg^{u}	36
	93 embryos Sprague-Dawley strain		One *in utero* injection on any one day from 12-16 days of gestation	No effect	50-125 μg/kg^{v}	76
Golden Hamsters						
	NS	PF	Daily oral dose, days 6-10 of gestation	No effect	<100 mg/kg^{t}	24
				Fetal death, teratogenic	20-100 mg/kg^{w}	24
	NS	PF	Single intravenous dose on day 8 of gestation	No malformed embryos, 9% resorption - Test 6% resorption - Control	2 mg/kg^{r}	47

Table 7 continued

Rabbit						
	NS	PF	Daily oral dose on days 6-18 of gestation	No effect	40 mg/kg[o]	36
Monkey						
	10, per group	PF	*Daily stomach tube dose from* 22-38 days of gestation	No effect	0.05, 1.0[g] or 10 mg/kg	32
	NS	PF	3 oral doses weekly for 4 weeks between days 20-48 of gestation	No effect on 12 fetuses removed by hysterectomy at 100 days gestation	5,10,20, 40[o] mg/kg	1
Sheep						
	NS	PF	Daily dose from 14-36 day of gestation	No effect	100 mg/kg[r,x]	11
	NS	PF	Dosed at various periods of gestation	No effect	113 mg/kg[r]	11

[a] NS - number of animals in study not stated or unavailable from literature source.

[b] PF - pregnant female

[c] 2,4,5-T acid, containing <0.1 mg/kg TCDD.

[d] s.c. - subcutaneous injection.

[e] 2,4,5-T acid containing 30 mg/kg TCDD.

[f] 2,4,5-T acid containing <0.02 mg/kg TCDD.

[g] 2,4,5-T acid containing 0.05 mg/kg TCDD

[h] Butyl ester of 2,4,5-T, containing <0.02 mg/kg TCDD.

[i] 2,4,5-T acid, containing 1.5 mg/kg TCDD.

[j] 2,4,5-T acid, technical grade, containing 0.5 mg/kg TCDD or analytical grade, containing <0.05 mg/kg TCDD.

[k] 2,4,5-T acid, containing <1.0 mg/kg TCDD.

[l] 2,4,5-T acid containing <0.05 mg/kg TCDD.

[m] 2,4,5-T acid, containing <0.1 mg/kg TCDD.

[n] Butoxyethylester of 2,4,5-T, containing <0.1 mg/kg TCDD.

[o] 2,4,5-T acid, containing 0.5 mg/kg TCDD.

[p] 2,4,5-T acid, containing <0.5 mg/kg TCDD.

[q] Butyl ester of 2,4,5-T, containing <0.5 mg/kg TCDD.

[r] 2,4,5-T acid, purity not stated.

[s] Butyl ester of 2,4,5-T, purity not stated.

[t] 2,4,5-T acid, free of TCDD. Detection level not stated.

[u] 2,4,5-T acid, containing 1.0 mg/kg TCDD.

[v] Purified 2,4,5-T acid, purity not stated.

[w] 2,4,5-T acid with oil, 0.5, 2.9 or 45 mg/kg TCDD.

[x] Propylene glycol butyl ester of 2,4,5-T.

of 2,4,5-T in susceptible animals varied with the content of the contaminant TCDD. Levels of TCDD greater than 1 mg/kg were required to enhance the embryotoxic and teratogenic potential of 2,4,5-T.

E. Carcinogenic and Tumorigenic Potentials of 2,4,5-T

The industrial production of 2,4,5-T always results in some TCDD contamination, although admittedly at very low levels (<0.01 ppm) with current technology. Nevertheless, in the following review, the effects of various levels of TCDD associated with the 2,4,5-T being tested must always be considered.

Innes et al (67) and the Bionetics Research Laboratories (12) reported that in groups of male and female mice receiving commercial 2,4,5-T (98 percent pure) there were no increases in any type of tumor in any group or combination of groups when compared to control animals. The treated mice were given 2,4,5-T at the dosage level of 21.5 mg/kg in 0.5 percent gelatin by stomach tube at seven days of age daily up to 28 days of age, followed by 60 mg/kg of diet until the mice were 78 weeks of age.

Muranyi-Kovacs et al (92) conducted a two month study in XVII/G and C3HF mice. Beginning at six weeks of age the mice were given 2,4,5-T (containing <0.05 ppm dioxins) in the drinking water at a dosage of 100 mg/l. Following the initial two month treatment the exposure was continued throughout the animals life span by mixing 2,4,5-T directlv with the diet at a concentration of 80 mg/kg. The average survival times for the XVII/G mice was 555 days in 20 treated males and 632 days in 19 treated females, compared to 516 days in 32 control males and 40 control females. No significant differences were found in the incidences of tumors in the XVII/G strain of mice between the treated and control mice. The XVII/G strain of mice have a known high spontaneous incidence of lung tumors. In test groups of 22 male and 25 female C3HF mice studied, the average survival times were 523 days in treated males and 621 days in treated females, compared to 641 days in 43 control males and 661 days in 44 control females. The total number of tumors was 13/22 in treated males and 13/15 in treated females, which was significantly different from that in the female controls of 9/44 (P<0.01). No significance was seen when test males with tumors were compared to control males with a tumor incidence of 22/43. The C3HF strain of mice has a known high spontaneous incidence of hepatomas.

In a 1968 study (12) groups of 18 male and 18 female mice from two different crossbred strains were given single subcutaneous injections of 98 percent pure, 2,4,5-T at a dosage level of 215 mg/kg in DMSO at 28 days of age and observed up to 78 weeks of age. Tumor incidences in treated mice of any groups or combination of groups were not significantly different from any groups or combination of groups of control animals that numbered 141, 154, 157 and 161. The control animals were either untreated or were injected with DMSO, 0.5 percent aqueous gelatin or corn oil.

Walker et al (141) demonstrated that six daily intraperitoneal injections of highly purified 2,4,5-T (99.0 percent) at the rate of 62 mg/kg effectively inhibited development of the Ehrlich ascites tumor being maintained in BALB/c mice. When the dosage of 2,4,5-T was increased to 80-85 mg/kg per day for six injections, the extent of inhibition of tumor development was doubled.

From data presented in Table 8 it appeared that 2,4,5-T was not carcinogenic in most strains of mice tested at the oral dosage ranges of 21.5 mg/kg or 60 to 100 mg/kg in the diet or drinking water. Single subcutaneous doses of 2.5 mg/kg 2,4,5-T did not induce tumor formation in mice and 62 to 85 mg/kg 2,4,5-T in six daily intraperitoneal injections actually inhibited Ehrlich ascites tumor development being maintained in BALB/c mice. The only exception noted was the results reported by Muranyi-Kovacs et al (92), where treated C3HF female mice had a significantly higher incidence of tumors than did the control females. These authors stated:

> The carcinogenesis observed in our experiments should be attributed to 2,4,5-T per se. Nevertheless, a problem in assessing the significance of this effect was the choice of statistical analysis. Since the average survival time was different in some experimental groups, the choice of the experimental animal in assessment of carcinogenic potential is very important. For practical reasons rodents, particularly mice, are often used without scientific justification for such a choice. The problem of species specificity in the metabolism of chemical carcinogens is a known variable.
>
> The work by Gehring et al [(49)] on 2,4,5-T showed that the kinetics of excretion of 2,4,5-T was extremely variable from one species to another. The half-life of 2,4,5-T in the plasma after a dose of 5 mg/kg was found to be 4.7 h in the rat, 77 h in the dog and 23 h in man. So the mouse being a rodent may not be the ideal experimental model for testing the carinogenicity of 2,4,5-T.

Muranyi-Kovacs et al (92) further noted that in their opinion 2,4,5-T should be placed in the C group of chemical substances whose activity has been insufficiently assessed and in C2 and C3 priority groups requiring additional data, implying that further testing in greater numbers of animals and in other species such as the rat and the dog was necessary.

F. Mutagenic and Cytogenetic Potentials of 2,4,5-T

As with 2,4-D, most of the mutagenic studies involving 2,4,5-T have been conducted in bacterial cultures or in plant and animal tissue cultures; however, Styles (133) investigated the cytotoxic effects of 2,4,5-T on *in vivo* and *in vitro* test systems and found no increase in

TABLE 8. Summary of literature data on the carcinogenic and tumorigenic potentials of 2,4,5-T in animals

Animal	Number Used	Route of Administration	Response	Dose	Reference
Mouse					
	18 M[a]/18 F[b] of two hybrid strains	Stomach tube, beginning at 7 days of age for 21 days, then in diet for 18 months	No effect	21.5 mg/kg[c] by stomach tube	12, 67
				60 mg/kg diet[c]	12, 67
	20 M/19 F XVII/G strain	Starting at 6 weeks of age for 60 days in drinking water, then in diet for life span	No effect	100 mg/l[d] for 60 days	92
				80 mg/kg diet[d]	92
	22 M/25 F C3HF	Starting at 6 weeks of age for 60 days in drinking water, then in diet for life span	No effect in males, more tumors treated in females than in controls	100 mg/l[d] for 60 days	92
				80 mg/kg diet[d]	92
	18 M/18 F	Single subcutaneous injections	No effect	215 mg/kg[e] in DMSO	12
	8 sex not stated BALC/c	Six daily intraperitoneal injections	Inhibited Ehrlich ascites tumor	62 mg/kg[f]	141
			Doubled inhibition	80-85 mg/kg[f]	141

[a] M - Male

[b] F - Female

[c] 2,4,5-T acid from a commercial source, TCDD level and purity not stated

[d] 2,4,5-T acid, containing <0.05 mg/kg TCDD

[e] 2,4,5-T acid, 98 percent pure, in dimethyl sulphoxide.

[f] 2,4,5-T acid, 99 percent pure, TCDD level not stated.

mutation rate and no evidence of mutagenicity in the test rats. He found serum from orally dosed rats was not mutagenic to *Salmonella typhimurium*. However, complete details of this study were not available.

Jenssen and Renberg (68) found there was not a detectable increase of micronuclei in the erythrocytes of mouse bone marrow after intraperitoneal administration of 100 mg/kg 2,4,5-T containing less than 1 mg/kg TCDD. Because of the high experimental resolution power of the test system used, it was particularly suitable for the detection of weak chromosome breaking activity of 2,4,5-T in mammal cells. The lack of penetration of 2,4,5-T into the cells was in accordance with the rapid excretion that is known to occur in the mammalian body. This experiment did not, in the authors opinion, constitute a reliable measure of the mutagenic potential of 2,4,5-T; however, in practice, the lack of penetration of this substance into the cells indicated it did not constitute a cytogenetic hazard to man.

In an abstract Buselmaier et al (19) reported on a large number of pesticides evaluated for mutagenic activity in mice with the host-mediated assay and to a smaller extent the dominant lethal method. These test systems took into account the mammalian metabolism and covered two different spectra of mutations: point mutations and the dominant lethal mutations which were thought to be the result of chromosomal aberrations. Back mutation systems of *Salmonella typhimurium* G46 His$^-$ and *Serratia marcescens* a21 leu$^-$ and *Serratia marcescens* a31 His$^-$ were used. In the host-mediated assay there was no significant increase in mutation rates after unspecified levels of subcutaneous injections of the acid or n-butyl ester of 2,4,5-T. All spot tests for this herbicide *in vitro* was also negative. When the n-butyl ester, unspecified purity, was given to test mice by a single intraperitoneal injection, at a dose of 100 mg/kg, no increases in dominant lethal mutations were seen.

Därving and Hultgren (30) reported that commercially available 2,4,5-T, with a TCDD concentration guaranteed on the label to contain less than 0.1 mg/kg, affected chromosomal and reproductive mechanisms in bone marrow cells from two different strains of mice. The authors concluded, however, that chromatid inter- or intra-exchanges were never observed. The study was not carried-out for sufficient time to demonstrate the effects on future generations of somatic cells.

Majumdar and Hall (85) investigated the effect of 2,4,5-T containing no detectable TCDD, on male and female Mongolian gerbils. Test animals ranging from 50-80 days of age were given 5 consecutive daily intraperitoneal injections of 50, 150, 250, 350 or 500 mg/kg. No effects were seen on the chromosomes of bone marrow cells at doses of 150 mg/kg or less. At levels of 250 mg/kg and above, significant increases in chromatid gaps, chromatid breaks and chromatid fragmentation were observed. No exchange figures or isochromosome gaps or breaks were reported.

Fujita et al (46) conducted studies to examine the cytogenetic effects of high purity 2,4,5-T (0.09 mg/kg TCDD) at levels of 10^{-7} to 10^{-14} M on human lymphocytes *in vitro*. Breaks, deletions and rings were observed. Chromatid breaks increased with increasing concentrations of 2,4,5-T; however, it was not possible to distinguish if this effect was due to cellular toxicity or to a potential genetic alteration.

Andersen et al (4) evaluated 110 herbicides for their ability to induce point mutation in one or more of 4 different microbial systems. The herbicide 2,4,5-T was included in this study. The authors did not state the purity of the compounds being tested. The 2,4,5-T did not cause point mutations in these microbial systems in comparison with known mutagens such as 5-bromouracil or 2-aminopurine. These observations of no mutagenicity of 2,4,5-T in *Escherichia coli* WP2 her^+ or her^- or in *Salmonella typhimurium* strains TA1535, TA1536, TA1537 or TA1538 were also confirmed in works by Nagy et al (94), Shirasu (136) and Shirasu et al (127).

A review of the literature on the mutagenic and cytogenic potentials of 2,4,5-T in animals generally supported the premise that 2,4,5-T, like 2,4-D, was not highly cytotoxic in laboratory animals. The herbicide did not increase mutation rates nor stimulate a mutagenic response in rats and mice. In various *in vitro* and *in vivo* test systems 2,4,5-T did cause chromatid aberrations in cultured human lymphocytes and affected the chromosomes and reproductive mechanisms in mouse and hamster bone marrow cells. It was not determined whether these affects were due to cellular toxicity or to a potential for genetic alteration of future generations of somatic cells. No mutagenic responses were seen in several studies using microbial systems for the detection of mutagenic and cytogenic responses to 2,4,5-T.

IV. REVIEW OF TCDD TOXICITY IN ANIMALS

A. The Acute and Short-Term Toxicity Potentials of TCDD

Studies on the extremely high acute toxicity of TCDD, the most toxic of the chlorinated dibenzo-*p*-dioxins, have been conducted by Carter et al (21), Greig et al (53), Gupta et al (56), Harris et al (59), King et al (76), Kociba et al (77), McConnell et al (87), Schwetz et al (121), Vos et al (140) and Zinkl et al (148).

Schwetz et al (121) noted that perhaps the most striking fact about TCDD was its ability to cause death after a single oral dose at levels as low as 0.6 μg/kg in male guinea pigs or 1000 μg/kg in the dog. Lethal doses to rabbits were in the same dose range with either oral (115 μg/kg), intraperitoneal (>252 μg/kg), or skin (275 μg/kg) administration. In mice, single oral doses of 1 to 130 μg/kg produced some deaths, however, no dose-response relationship was established. Schwetz et al (121) noted that approximately half the deaths in mice occurred between 13 and 18 days after treatment.

Poland and Kende (108) considered TCDD to be one of the most potent low molecular weight toxins and teratogens known. They noted that most poisons act rapidly and kill by impairing the physiologic function of the nervous system. TCDD in contrast, is a "cellular poison." In the rat, deaths appeared to have resulted from hepatic necrosis and ensued weeks after a single oral dose.

Harris et al (59), Schwetz et al (121), and Vos et al (140) also noted TCDD produced hepatic cell necrosis that was the probable cause of death in the rats in their studies. They also noted that in mice and guinea pigs, hepatic cell necrosis and liver insufficiency occurred only minimally.

Putnam and Courtney (109) treated female Wistar rats with single oral doses of 100 μg/kg TCDD and found it caused a biphasic decline in body weight with a cessation of food and water consumption and urine production. The first phase started immediately after dosing and lasted 7-10 days followed by a recovery from 4-6 days during which time the rats ate and drank and regained about 10-15 percent of their body weight. This was followed by a second phase which occurred at 16-24 days after treatment with a weight loss of about 15-30 percent. If the loss of body weight exceeded 30 percent the rats usually died. Daily administration of water, electrolyte solution, or a balanced liquid diet did not alter or reverse the biphasic response.

Cunningham and Williams (28) treated groups of 12 to 16 weanling male Wistar rats with single oral doses of 0 or 10 μg/kg TCDD. This was close to the lethal dose for when this amount was given orally to rats each day in a preliminary experiment, all died within 2 to 4 days. The lowest level in a single dosage that caused an increase in liver weight of rats in the preliminary study was 0.1 μg/kg. The TCDD had no effect on the rate of incorporation of ^{3}H-acetate into liver lipids; however, it may have restricted the transport of lipids out of the liver. The storage of lipids reached a maximum at about 3 days after the TCDD was given and was accompanied by a significant increase in the incorporation of ^{14}C-leucine into liver proteins. The increased synthesis of all proteins may have resulted from an induction of liver enzymes by TCDD.

Harris et al (59) found the mortality pattern was very near the same in rats and guinea pigs when TCDD was given as a single oral dose or divided into daily or weekly doses over a 4 to 5 week period. He noted that this mortality pattern could be interpreted as demonstrating a cumulative toxicity from the TCDD.

In rats, guinea pigs and mice, changes in the weight of the thymus appeared to be the most sensitive indicator of TCDD exposure according to work by Harris et al (59). These decreases in thymus weight occurred with doses of TCDD that had no effect on body weight.

Van Miller et al (137) produced high levels of TCDD in the skin of rhesus monkeys by giving a single intraperitoneal injection of 400 μg/kg TCDD and produced clinical signs of alopecia and acne.

A summary of the literature on the LD_{50} levels of the acute toxicity of TCDD for animals is presented in Table 9. TCDD was found to be an extremely toxic compound with an oral LD_{50} range of 0.6 µg/kg in male guinea pigs to 115 µg/kg male and female rabbits. Male rats appeared to be more sensitive than females when TCDD toxicity was studied in the Sherman (Spartan) strain rat. In rabbits, essentially similar dosage levels of TCDD caused death following either intraperitoneal, oral or skin administration. Limited studies on dogs suggested that they were less sensitive to TCDD than were the other laboratory animal species studied. In all species studied however, reduction in body weight was a common finding following TCDD treatment while other signs of toxicity were species dependent.

B. The Subacute and Chronic Toxicity Potentials of TCDD

Subacute and chronic doses of TCDD produced a variety of toxic effects, including hepatic necrosis, thymic atrophy and lesions of the myocardium in rats (20, 56), thymic atrophy, depletion of lymphoid organs and hemorrhage and atrophy of adrenal zona glomerulosa in guinea pigs (56) and hepatic necrosis in rabbits (120). The main target organs of TCDD in rats, guinea pigs and mice appeared to be the liver and thymus (56, 69, 70, 139, 140). The degree of hepatic involvement appeared to be dose dependent and the severity of the changes produced varied between species (56). A single oral dose of 126 µg/kg TCDD resulted in loss of body weight and death with an enlarged fatty liver after 21 days in C57B1/6 mice. A progressive necrotic centilobular liver lesion was seen (71).

Vos and Moore (139) studied pre- and postnatal effects of TCDD in groups of 5, 6 and 5 pregnant C57B1/6 mice dosed at 0, 2 or 5 µg/kg TCDD on days 14 and 17 of gestation and postnatally on day 1, 8 and 15. All neonates were weaned on day 23 and used for a skin graft experiment. This treatment resulted in a severe depletion of lymphocytes in the thymic cortex of the offspring. Cellular immunity was impaired and allograft rejection times were prolonged.

Murray et al (93) conducted a three generation reproduction study to evaluate the effects of chronic, low-level ingestion of TCDD in Sprague-Dawley rats administered daily doses of 0, 0.001, 0.01 or 0.1 µg/kg provided via the diet for 90 days. No signs of toxicity were noted in either male or female rats during the TCDD feeding study.

Vos et al (140) found the most significant findings in both mice and guinea pigs treated with sublethal doses of TCDD were in the lymphoid system where there was a supression of cell mediated immunity at doses of 2 and 5 µg/kg TCDD.

Thigpen et al (134) found that low levels of TCDD did not produce overt clinical or pathological changes, however, these low levels

TABLE 9 . Summary of literature data on the no-effect, LD_{50} and LD_{100} levels of the acute toxicity of TCDD for animals

Animal	Number Used	Route of Admin.	Dose-Toxicity	Single Dose µg/kg	Reference
Mouse	10 CD-1 strain C57Bl/6Sch strain	Oral	LD_{100}	>50	59
	NS[a]	Oral	A few sporadic deaths	1-130	121
	29 M[b] C57Bl/6 strain	Oral	LD_{100}	150	50
	M NS	Intraperitoneal	LD_{50}	120[c]	138
Rat	5 M	Oral	No effect	8	121
	5 M	Oral	No effect	16	121
	10 M	Oral	LD_{100}	32	121
	25 M Sherman (spartan) strain	Oral	LD_{50}[d]	22	121
	NS F Sherman (spartan) strain	Oral	LD_{50}[d]	45	121

Table 9 continued

Guinea Pig						
	NS	M	Oral	LD_{50}[e]	.6	121
	NS	M		LD_{50}[e]	2.1	121
	Hartley strain					
Rabbit						
	NS	M/F	Oral	LD_{50}[e]	115	121
	5	M/F	Topically to skin	LD_{50}[e]	275	121
	5	M/F	Intraperitoneal	No effect	32	121
	5	M/F	Intraperitoneal	2 of 5 died	>252	121
	5	M/F	Intraperitoneal	3 of 5 died	500	121
	New Zealand albino					
Dog						
	2	M	Oral	No effect	300	121
	2	M	Oral	LD_{100}	3000	121
	2	F	Oral	No effect	30	121
	2	F	Oral	No effect	100	121
	Beagles					
Monkey						
	1	F	Oral	LD_{50}[f]	<70	87
	Rhesus					

[a] NS - Number of animals in study not stated or unavailable from literature source

[b] M - Male

[c] ^3H-TCDD

[d] A calculated LD_{50}

[e] Responses to individual doses when LD_{50} could not be calculated

[f] Correlated the acute LD_{50} of TCDD with the clinical and pathological manifestations - not true calculated LD_{50}

reduced host defenses. When 1 µg/kg was given orally once a week for 4 weeks to mice before infection with *Salmonella bern*, an increased mortality and decreased time from infection to death was noted.

Weissberg and Zinkl (142) and Zinkl et al (148) noted hematological changes in mice, rats and guinea pigs treated with TCDD including lymphopenia and thrombocytopenia at dosage rates of 0.004 to 10 µg/kg for various repeated doses.

Goldstein (50) gave TCDD orally to mice once a week over a 4 week period at a dosage of 25 µg/kg and found a 2,000-fold increase in the levels of 8- and 7-carboxyporphyrins in the liver.

In a 13-week feeding study by Kociba et at (77), Sprague-Dawley rats of both sexes were given 0.001 or 0.01 µg/kg TCDD five days per week. A slight increase in relative liver weight occurred in those animals receiving 0.01 µg/kg TCDD. A steady state concentration of TCDD was attained in body tissues by the end of the study.

Vos and Moore (139) in a pre- and postnatal study, treated groups of 5, 4 and 6 pregnant Fisher-334 rats with 0, 1 or 5 µg/kg TCDD prenatally on days 11 and 18 of gestation and postnatally on days 4, 11 and 18 via gastric intubation. Most of the neonates in the 5 µg/kg group died. Only the spleens of 25-day-old male animals from the 0 and 1 µg/kg groups were used for immunologic studies. At 1 µg/kg the pups had a depressed body and spleen weight. At 5 µg/kg, in those pups that survived, the body and spleen weights were depressed and the thymus was severely affected with marked depletion of lymphocytes in the thymic cortex. Cellular immunity was impaired with allograft rejection times being prolonged.

Schwetz et al (121) found that solutions of 0.04 µg TCDD/ml of benzene was acnegenic in a rabbit ear bioassay study where the solution was applied to the inside of the ear 5 days per week for four weeks.

Norback and Allen (98) fed fat, containing unspecified concentrations of chlorinated dibenzo-*p*-dioxins in the diet, to *Macaca mulatta* monkeys for 100 days and found the monkeys developed alopecia, subcutaneous edema, anemia, progressive leukopenia and hypoproteinemia. Enlargement of the liver, hydropericardium, gastric hyperplasia and ulceration as well as hyperplasia of the lymph tissue and bone marrow was noted in the treated monkeys.

Allen et al (2) found that female rhesus monkeys given a diet containing 500 ng/kg TCDD for 9 months became anemic within 6 months and pancytopenic after 9 months of exposure. Marked thrombocytopenia was associated with widespread hemorrhage. Death occurred in five of the eight animals between months 7 and 12 of the experiment at total exposure levels of 2-3 μg/kg TCDD body weight. At autopsy, in addition to the hemorrhage, there was a distinct hypocellularity of the bone marrow and lymph nodes. Death of these monkeys was attributed to complications from the severe pancytopenia.

McNulty (89) fed one rhesus monkey a diet containing 2 μg/kg TCDD and another monkey a diet containing 20 μg/kg TCDD. The first animal died within 76 days, while the second animal died in 12 days. McNulty noted that although responses in two animals scarcely provided data for a dose-response curve, two conclusions could be drawn: (a) a total TCDD dose of less than 10 μg/kg of body weight accumulated over a few weeks period, and (b) young rhesus monkeys were among the most TCDD-susceptible animals of those that have been tested.

A summary of literature data on the subacute and chronic toxicity of TCDD in animals is presented in Table 10. Subacute and chronic doses of TCDD produced a variety of toxic effects, including hepatic necrosis in mice, rats and rabbits; thymic atrophy in mice, rats and guinea pigs with adrenal gland hemorrhages and depletion of lymphoid organs also being seen in guinea pigs. Repeated oral doses of 0.001 to 10 μg/kg TCDD for four to 13 weeks did not significantly affect weight gain nor were signs of toxicity noted in mice and rats. Suppressed immune responses and changes in liver enzymes were noted, however, in mice. Repeated doses of TCDD as low as 1 μg/kg caused guinea pigs to become moribund and repeated doses of 0.04 μg/kg decreased lymphocyte counts. Rabbits developed acne of increasing severity when doses of 0.04 to 400 μg/kg were applied repeatedly to the internal surface of the ear. A total oral dose of 2-3 μg/kg over a nine month period produced severe hematological changes and death in rhesus monkeys.

C. Absorption, Distribution and Excretion of TCDD

Following a single oral administration of 50 μg/kg ^{14}C-TCDD to rats, Piper et al (102, 103) found that almost 30 percent was eliminated in the feces during the first 48 h. The half-life for the disappearance of ^{14}C activity from the body was 17.4 ± 5.6 days. After this time the excretion of ^{14}C activity via the feces was from 1-2 percent per day. As the ^{14}C-TCDD was absorbed into the body tissues most of the activity was localized in the liver and fat at levels about 10 times higher than that in other tissues. A total of 53.2 percent of the dose was eliminated via the feces and 13.2 percent via the urine, while 3.2 percent was expired into the air when measured over a 21 day period.

Rose et al (114) found that, following daily oral administration of 0.01, 0.1 or 1.0 μg/kg ^{14}C-TCDD five times per week for seven weeks to Sprague-Dawley rats, the major route of excretion was via the feces. Urine contained 3-18 percent of the cumulative dose of ^{14}C activity after the seven week treatment. The half-life of ^{14}C activity in the rats studied was 23.7 days.

TABLE 10. Summary of literature data on the subacute and chronic toxicity of TCDD in animals

Animal	Number Used	Route of Administration	Effect	Dose	Reference
Mouse					
	377 M^a C57Bl/6JFh (J67) strain Specific - Pathogen free	Once per week by gastric tube for 4 weeks	No effect on weight gain	0.5, 1, 5 and 10 µg/kg	134
			Significant decrease in weight gain	20 µg/kg	134
			No effect on mice challenged with Herpesvirus suis	0.5, 1, 5, 10 and 20 µg/kg	134
			No effect on mice challenged with Salmonella bern	0.5 µg/kg	134
			Significant increase in mortality of mice challenged with Salmonella bern	1 µg/kg and greater	134
	5-6 per group C57Bl/6Sch F^b strain C57Bl/6 M^a strain	Oral dose given days 14 and 17 of gestation and post-natally on day 1, 8 and 15	No effect on weight gain	2 µg/kg	140
			Suppressed cellular immunity	2 or 5 µg/kg	140
	NS^c F CD-1	Single oral dose after 8 weeks of age	Hematological changes at 1 week after dose; normal at 3 weeks	1, 10 or 50 µg/kg	148
	12 M C57Bl/6 strain	Oral dose once per week for Four weeks	2000 fold increase in carboxyporphyrins in the liver	25 µg/kg	50

Table 10 continued

Rat					
	NS M/F Sprague-Dawley strain	Daily oral dose for 90 days	No signs of toxicity	0.001, 0.01 or 0.1 μg/kg	93
	NS M/F Sprague-Dawley	Daily oral dose, 5 days per week for 13 weeks	No toxicity, slight increase in relative liver weight at 0.01 μg/kg	0.001 or 0.01 μg/kg	77
	NS F CD strain	Daily oral dose for 30 days	Liver enzyme changes and hematological changes	10 μg/kg	148
Guinea Pig					
	NS F Hartley strain	Weekly oral doses for 8 weeks	Moribund at 3 to 5 weeks	1.0 μg/kg	148
			Significant decrease in lymphocyte counts	0.04 μg/kg	148
Rabbit					
	NS	Applied to inside of ear, 5 days per week for 4 weeks in a .1 ml volume	Acne with increasing severity as dose was increased	0.04 to 400 μg/kg	121
Monkey					
	NS Macaca mulatta	Fed fat containing 64% mass tetrachlorinated compounds in diet for 100 days	Multiple toxic signs	Unknown	98
	8 F Macaca mulatta	Fed in diet for 9 months	Hematologic changes, 5 animals died	500 ng/kg of diet 2-3 μg/kg total exposure	2

Table 10 continued

2 *Macaca mulatta*	Fed in diet	Death in 12 days	20 μg/kg diet	89
		Death in 76 days	2 μg/kg diet	89

[a] M - Male

[b] F - Female

[c] NS- Number of animals in study not stated or unavailable from literature source

Allen et al (2) treated 40 male Sprague-Dawley rats with a single intragastric dose of 50 µg/kg of ^{14}C-TCDD. One-half of the animals died within 25 days, 25 percent being accounted for during the first 3 days. The total amount of radioactivity in the urine was 4.5 percent of the total dose, with the highest daily levels being excreted toward the end of the experiment. A large percentage of the remaining radioactivity was localized in the liver and of this over 90 percent was located within the microsomal fraction.

Fries and Marrow (44) found the half-life to be 12-15 days in rats given 7 or 20 µg/kg ^{14}C-TCDD of diet (equivalent to 0.5 or 1.5 µg/kg per day) for 42 days.

Vinopal and Casida (138) administered ^{3}H-TCDD by a single intraperitoneal injection to male mice at the LD$_{50}$ dose of 120 µg/kg and found that it was not measurably converted to water soluble products and was eliminated primarily in the feces. Traces of tritium activity were detected in the urine. A large proportion of the administered dose persisted in the unmetabolized form in the liver, partially concentrated in the microsomal fraction for 11 to 20 days after treatment. The ^{3}H-TCDD was not metabolized by liver microsomal fractions from mice, rats or rabbits.

Gasiewicz and Neal (48) studied the tissue distribution and excretion of ^{14}C-TCDD in adult male guinea pigs for up to 15 days following its intraperitoneal injection of 2.0 µg/kg. On day 1 the highest levels of radioactivity were located in the adipose tissue 2.36 percent, adrenals 1.36 percent, liver 1.13 percent, spleen 0.70 percent, intestine 0.42 percent and skin 0.48 percent. All other tissues examined contained less than 0.3 percent. The level of ^{14}C-TCDD in the liver increased to 3.23 percent on day 15. An increase in ^{14}C-TCDD was also noted in the adrenals, kidneys and lungs while adipose tissue and skin decreased in radioactivity. For the 15 days of the experiment the total urinary and fecal excretion of radioactivity was less than 1 and 5 percent respectively. The effects of 1.0 µg/kg TCDD upon plasma levels of Na, K, Cl, CO$_2$, Fe, Ca, inorganic P, alkaline phosphatase, SGOT, SGPT, LDH, glucose, urea nitrogen, creatinine, uric acid, total protein, albumin, cholesterol, triglycerides and bilirubin were determined periodically up to 14 days and compared to pair-fed control animals. Statistically significant increases in plasma albumin, total protein, Fe, urea nitrogen, cholesterol and triglycerides were observed in the TCDD-treated guinea pigs.

The primary route of excretion for TCDD in animals appeared to be the feces, with urinary excretion occurring at a much reduced rate. Liver and fat accumulated about 10 times higher levels of TCDD than did other body tissues. The half-life for TCDD in rats following a single or repeated exposure was 12-24 days after termination of treatment. Large proportions of an administered dose of TCDD remained unmetabolized in the liver microsomes and were slowly excreted over an extended period.

D. Embryotoxic, Fetotoxic and Teratogenic Potentials of TCDD

The embryotoxic and teratogenic effects of TCDD in mice have been described by Courtney and Moore (27), Neubert and Dillmann (96), Neubert et al (97), and Smith et al (128) where doses as low as 1-10 μg/kg, given in a single or repeated dose, caused significant increases in the frequency of cleft palate and kidney anomalies.

Neubert (95), and Neubert et al (97) noted a dose-response relationship for producing cleft palates in mice with TCDD. They also observed increased incidences in the frequency of cleft palate in mice, apparently caused by the synergistic effect of combining 'sub-threshold' and 'threshold' levels of TCDD with similar low levels of other known teratogens such as the weak teratogen 2,4,5-T when administered during days 6-15 of gestation.

Moore et al (91) confirmed that exposure to TCDD via the milk was a major factor in the development of renal hydronephrosis in mouse pups when the nursing dam received a single oral dose of 1, 3 or 10 μg/kg TCDD at parturition. This effect was also seen in mouse pups nursed by a foster mother treated with TCDD during pregnancy or at the time of parturition. The common etiology of these kidney anomalies, whether prenatal or postnatal, was TCDD interference with development of the metanephric kidney and/or subsequent maturation. The incidence and degree of hydronephrosis was a function of dosage and length of target organ exposure.

The dose effecting 50 percent of the test organisms (ED_{50}) for cleft palate production in NMRI mouse pups was estimated by Neubert et al (97) to be 40 μg/kg TCDD per day. The nc effect level during days 6 to 15 of gestation was estimated at 2 μg/kg TCDD per day with no pronounced fetal mortality occurring when 3 μg/kg TCDD was given from day 6 to day 15 of gestation.

Becker (8) has concluded that the influence of a teratogenic substance closely related to the critical developmental period of a particular tissue or organ. After this critical period passed, damage to other tissues may have occurred even if no significant malformations were observed. Unspecified doses of TCDD produced an extremely fatty degeneration of the liver in adult female rats when they were treated on days 13 to 15 of gestation. Fatty inclusions were seen in the liver of embryos from these treated females; however, no structural anomalies were noted in any of the embryo livers.

Courtney and Moore (27) produced cleft palates in three strains of mice by giving 1 or 3 μg/kg TCDD subcutaneously on days 6 to 15 of pregnancy, while Courtney (25) found TCDD to be the most fetotoxic and teratogenic of several dioxin compounds when given at 25, 50, 100, 200, and 400 μg/kg per day orally and 25, 50, 100, 200 μg/kg per day subcutaneously in CD-1 mice on days 7 to 16 of pregnancy. Fetal mortality increased with the dose: up to 97 percent in orally treated dams and up to 76 percent in dams receiving subcutaneous administration of the highest levels of TCDD. Other anomalies observed were hydrocephalus, lack of eyelid formation (open eye) and clubfoot with edema and internal hemorrhages being noted in fetuses of dams receiving the highest doses.

Smith et al (128) administered 0.001, 0.01, 0.1, 1.0 and 3.0 μg/kg TCDD per day to CF-1 mice by gavage from days 6 to 15 of pregnancy. Only at the 1.0 μg/kg dose was the percentage of resorption sites per implantation sites significantly higher than in the control animals. At 3.0 μg/kg, cleft palate occurred in 71 percent of the treated litters and at 1.0 μg/kg, 21 percent had cleft palate. Renal anomalies occurred in 28 percent of the litters treated at 3.0 μg/kg and in 5 percent of the litters treated at 1.0 μg/kg. No significant anomalies were seen at the other dosage levels.

Embryo lethal effects have occurred in rats under experimental conditions imposed by Sparschu et al (131). Courtney and Moore (27) have observed kidney anomalies in rats, while Khera and Ruddick (75) observed intestinal hemorrhages and general edema in rat fetuses when oral or subcutaneous doses ranging from 0.03 to 16.0 μg/kg TCDD were administered daily to dams on days 6 to 15 of gestation.

Sparschu et al (131) administered 0.03, 0.125, 0.5, 2.0 and 8.0 μg/kg TCDD per day to Sprague-Dawley rats on days 6 to 15 of gestation. At 8.0 μg/kg per day all fetuses were resorbed. Fetal weights were significantly ($p<0.05$) depressed at the 0.125 and 2 μg/kg per day level. Internal hemorrhages were observed at the 0.125, 0.5 and 2.0 μg/kg per day level. No adverse effects were noted in the fetuses of dams treated at the 0.03 μg/kg per day level. The authors suggested that 0.03 μg/kg per day was the no effect level for fetal and embryotoxic effects in rats.

Khera and Ruddick (75) studied the perinatal effects of TCDD in Wistar rats in a two part experiment by giving daily oral doses of 0.125, 0.25, 0.5 and 1.0 μg/kg TCDD on days 6 to 15 of pregnancy. Visceral lesions were observed at 0.25 μg/kg per day and above with slight decreases in fetal weight also being observed. Postnatal effects of prenatal exposure to TCDD were studied by allowing offspring of treated dams to be reared by untreated dams until weaning. At maternal levels of 0.5 and 1.0 μg/kg per day, reduced survival, lowered body weight and reduced reproductive ability in the offspring were observed. At levels of 0.125 μg/kg per day no fetotoxic effects were observed.

In the second part of the Khera and Ruddick study (75), rats were treated with daily oral doses of 1, 2, 4, 8 and 16 μg/kg TCDD on days 6 to 15 of pregnancy. At doses of 1.0 and 2.0 μg/kg per day, visceral lesions, reduction in fetal weight, and lowering of the number of live fetuses per litter were observed. Doses of 1 μg/kg per day or more produced maternal toxicity with all doses of 4 μg/kg or more producing 100 percent embryo lethality. The fetotoxic no effect level in Wistar rats appeared to be 0.125 μg/kg per day with any level of 0.25 μg/kg per day or more on days 6 to 15 of pregnancy adversely affecting fetal rat development.

Courtney and Moore (27) administered TCDD to CD rats at the rate of 0.5 μg/kg per day subcutaneously in solutions of 100 percent DMSO on days 6 to 15 of gestation. Kidney anomalies were seen in 67 percent of the litters of treated females. At this level, TCDD did not affect fetal mortality or fetal weight, nor were cleft palates observed in any of the fetuses.

A summary of literature on the embryotoxic, fetotoxic and teratogenic potentials of TCDD in animals is presented in Table 11. It was apparent that TCDD caused birth defects and embryo mortality. Repeated daily oral doses of 0.1 to 2 μg/kg in pregnant mice produced no effect on the embryos; however, 3 μg/kg was the threshold level for production of cleft palate and kidney abnormalities. Single or repeated oral doses of 6.5 to 40 μg/kg TCDD were required to produce cleft palate in 50 percent or more of some strains of mouse embryos being studied. Daily subcutaneous injections of 1 to 3 μg/kg TCDD produced cleft palate and kidney abnormalities in 50 percent or more of three different strains of mouse embryos studied. Repeated oral doses of 25 to 400 μg/kg TCDD produced increasing fetotoxic and teratogenic responses in mice. Repeated daily oral doses of 0.03 to 0.125 μg/kg TCDD produced no effect in some strains of rat embryos while repeated oral doses of 0.125 to 2 μg/kg TCDD depressed fetal weight, lowered fetal survival and caused internal hemorrhages in fetuses. Repeated daily oral doses of 4 to 8 μg/kg TCDD produced 100 percent fetal mortality in rats. Signs of embryo toxicity and fetal death occurred in rats more frequently than did any signs of teratogenicity. When teratogenic lesions did appear in rats, kidney abnormalities were more common than cleft palate.

E. Carcinogenic and Tumorigenic Potentials of TCDD

In a preliminary report by Toth et al (135), 50 ten-week old male random bred Swiss H/Riop mice received gastric intubations of 7 μg/kg TCDD in sunflower oil for 12 months. No tumors were observed in 19 mice receiving post mortem examinations at the end of the treatment period. The livers from three animals showed histological evidence of cirrhosis and eight animals had developed dermatitis and showed histological evidence of increased amyloid in the tissues. Weekly doses of 0.007 and 0.7 μg/kg TCDD were given for 12 months to similar groups of mice. No pathological lesions were observed in five animals killed two months after the end of treatment. All surviving mice were kept for life-span studies and observation for the development of tumors.

TABLE 11. Summary of literature data on the embryotoxic, fetotoxic and teratogenic potentials of TCDD in animals

Animal	Number Used	Route of Administration	Response	Dose µg/kg	References
Mouse					
	700 total/PF^{a} NMRI strain, 7000 fetuses examined	Daily oral-dose, days 6-15 of gestation	No effect	2 (estimated)	96
			CP^{b} - Threshold	3^{c}	96
			CP - ED$_{50}$^{d}	6.5	96
		Daily oral dose, days 9-13 of gestation	CP	9^{c}	96
			CP - ED$_{50}$^{d}	<9	96
	100 total/PF	Single oral dose, day 13 of gestation	CP	15^{c}	97
			CP - ED$_{50}$	40	97
		Single oral dose, day 11 of gestation	CP	5^{c}	97
			CP - ED$_{50}$	15	97
	35 litters total from CD-1, DBA/2J, and C57Bl/6J strains	Daily doses given subcutaneously on days 6-15 of gestation	CD-1 - CP effect, 1 litter only	1^{c}	27
			- CP, Threshold	3^{c}	27
			- CP, ED$_{50}$	>3	27
			- KA^{e}, Threshold	1^{c}	27
			- KA, ED$_{50}$	1 - 3	27
			DBA/2J - CP, Threshold	3^{c}	27
			- CP, ED$_{50}$	>3	27
			- KA, Threshold	3^{c}	27
			- KA, ED$_{50}$	>3	27
			C57Bl/6J - CP, Threshold	3^{c}	27
			- CP, ED50	>3	27
			- KA, Threshold	3^{c}	27
			- KA, ED50	<3	27

Table 11 continued

	31 PF CD-1 strain	Daily oral dose, days 7-16 of gestation	Fetotoxic, teratogenic, increasing w/dosage up to 97% at highest dose	25, 50, 100, 200, and 400	25
		Daily dose given subcutaneously on days 7-16 of gestation	Fetotoxic, teratogenic, increasing w/dosage up to 76% at highest dose	25, 50, 100, and 200	25
	17 PF	Daily oral dose, by gavage, days 6-15 of gestation	No effect	0.1	128
	19 PF		Increased fetal resorption sites, 21% CP, 5% KA	1	128
	14 PF		71% CP, 28% KA	3	128
	18 litters[f]	Single oral dose, day 10 of gestation	No effect, CP 34% KA	1	91
	16 litters[f]	Daily oral dose, days 10-13 of gestation	1.9% CP 58.9% KA	1	91
	14 litters[f]	Daily oral dose, days 10-13 of gestation	55.4% CP 95.1% KA	3	91
	NS[g]/fetuses	Females given oral dose at parturition	Renal hydronephrosis, 12, 71 or 75% depending on dose	1, 3 or 10	91
Rat					
	51 total/PF Sprague-Dawley (Spartan) strain	Daily oral dose, days 6-15 of gestation	No effect	0.03	131
			Depressed fetal weight	0.125 and 2	131
			Internal hemorrhages in fetuses	0.125, 0.5 or 2	131
			All fetuses died	8	131

Table 11 continued

103 total/PF	Daily oral dcse, days 6-15 of gestation	No effect	0.125	75
		Slight decrease in fetal weight	0.25	75
		Reduced fetal survival, lower body weight and lowered reproductive ability in progeny	0.5 and 1	75
		Visceral lesions, reduced fetal weight, increased fetal death with maternal toxicity	1 and 2	75
		100% embryo death	4	75
6 PF 48 fetuses CD strain	Daily subcutaneous dose, days 6-15 of gestation	No effect on fetal mortality or CP 67% KA	0.5	27

[a] PF - Pregnant Female

[b] CP - Cleft palate

[c] Lowest dose with which a significant teratogenic effect has been produced. In some cases this is the only dose level tested and does not necessarily represent the lowest dose which could result in teratogenic effects.

[d] ED_{50} - Dose required to produce an effect in 50% of animals

[e] KA - Kidney abnormalities

[f] C57Bl/6 strain

[g] NS - Number of animals in study not stated or unavailable from literature source

[h] se given in 100 percent dimethylsulfoxide solution (DMSO)

Van Miller et al (136) recently reported the results of a two year study where ten groups of 10 male Sprague-Dawley rats were fed a laboratory diet containing 0, 1, 5, 50, 500 or 1,000 μg/kg TCDD of food or 1, 5, 50 or 500 ng/kg TCDD of food for 78 weeks. All rats receiving the 50, 500 or 1,000 μg/kg TCDD of food died between the second and fourth week of treatment. In seven remaining groups, only one animals died before the 30th week and that death occurred in the 500 ng/kg TCDD of food at the 17th week. In the 1 and 5 μg/kg TCDD of food groups, all animals died between the 30th and 90th weeks of the experiment. The number of animals dead at the 95th week of the experiment were: 0 dose 6/10, 1 ng/kg 2/10, 5 ng/kg 4/10, 50 ng/kg 4/10, 50 ng/kg 4/10 and 500 ng/kg 5/10. Those animals surviving after the 95th week were killed and subjected to complete necropsy examinations. In all rats surviving past the 65th week laparotomies were performed and biopsies of any tumors were taken. After the 78th week on treated diets, the rats were placed on the same diet used to feed the control animals. Tumorigenic and toxic effects were observed in rats from the lowest six dosage groups. The overall incidence of neoplasms in these six experimental groups was 23/60 (38 percent) compared with 0/20 (0 percent) in both the 1 ng/kg and the control groups. Neoplastic nodules and cholangiocarcinomas of the liver were observed in 40 percent of the rats ingesting 5 μg/kg TCDD of food; two animals had both neoplastic nodules of the liver and cholangiocarcinomas.

Van Miller et al (136) also found that tumors developed in 24/50 (46 percent) of the rats ingesting 5, 50 or 500 ng/kg TCDD of food and 1 or 5 μg/kg TCDD of food, compared to none (0/10) in the control animals. The tumors seen were carcinomas of the ear duct, kidney and liver. Three retroperitoneal histiocytomas were described as metastasizing to the "lungs, kidney, liver and skeletal musculature." Three of the ten deaths which occurred in the 5 μg/kg TCDD of food dose group were attributed to aplastic anemia. One animal in the 500 ng/kg TCDD of food group had a severe liver infarction.

Kociba et al (78) conducted a chronic study of TCDD toxic effects to Sprague-Dawley rats fed 0.1, 0.01 or 0.001 μg/kg TCDD daily for two years to groups of 50 rats of both sexes. Eighty-six animals of each sex served as controls. Discernible increases were noted in the incidence of hepatocellular carcinomas of the liver and of squamous cell carcinomas of the lung, hard palate/nasal turbinates and tongue in rats fed at the rate of 0.01 μg/kg. They also reported decreased incidences of pituitary, uterine, mammary gland, pancreatic and adrenal gland tumors at the 0.01 μg/kg level. The squamous cell carcinoma of the hard palate observed in one female rat receiving this dose was considered unrelated to TCDD treatment since a similar tumor had occurred in other unrelated studies. At 0.001 μg/kg TCDD, no significant lesions were seen in male rats and the only lesion of significance in female rats at the 0.001 μg/kg TCDD was swollen hepatocytes, considered to be a reversible lesion.

Many chemically nonreactive carcinogens are enzymatically converted to biologically active carcinogens. The enzyme aryl hydrocarbon hydroxylase (AHH) has been strongly implicated in this process (86). Kouri et al (82) studied AHH induction in human lymphocyte cultures by TCDD. The authors stated:

> TCDD itself is not a potent carcinogen in mice; however, the synergistic action of TCDD with 3-methylcholanthrene (MC) produces cancer in different strains of mice in direct proportion to the degree of elevation of the induced hydroxylase activity and associated cytochrome p_1-450 content.

Their study showed a positive correlation between basal enzyme activity and enzyme levels maximally inducible by either TCDD or MC. They also found that TCDD was about 40 to 60 times more potent than MC as an inducer of hydroxylase activity in cultured human lymphocytes.

The implication of TCDD in AHH inducibility had also been reported by Poland and Glover (105, 106) and Poland et al (107) in their studies on chick embryo livers. They found that all dioxins which were potent inducers have halogens at three of the four lateral ring positions and at least one non-halogenated carbon atom. When TCDD potency, as an inducer of hepatic AHH activity, was compared with that of MC by a computer bioassay technique, data reflected that TCDD may be 28,640 times as potent as MC on a molar basis.

Allen et al (2) conducted a study in which female rhesus monkeys were fed diets containing 500 ppt TCDD for nine months. Anemia, thrombocytopenia and leukopenia were the most debilitating changes noted. The altered lymphopoiesis could be associated with immune suppression. Epithelial changes, including hypertrophy, hyperplasia, and metaplasia were reported in these TCDD exposed monkeys.

A summary of the literature on the carcinogenic and tumorigenic potentials of TCDD in animals is presented in Table 12. It was noted that in a preliminary study where 0.007, 0.07 and 7 μg/kg TCDD was given in weekly oral doses for 12 months to mice, no tumors were produced. In rats, levels of 1 and 5 μg/kg TCDD of diet and 1, 5, 50 and 500 ng/kg TCDD of diet fed for 78 weeks produced an overall tumor incidence of 38 percent in the test animals. At 0.001 μg/kg TCDD, given via the diet to rats for 2 years, no effect was produced. A level of 0.01 μg/kg TCDD given via the diet to rats, for 2 years, produced liver nodules and hyperplasia of the epithelium of the lungs. An increase in liver and lung carcinomas was seen when 0.1 μg/kg TCDD was fed to rats for 2 years via the diet. An interesting unexplained observation, however, was the reduction of pituitary, uterine, mammary, pancreas and adrenal tumors. Monkeys fed 500 ng/kg TCDD of diet for 9 months did not develop tumors but died of marked hematological alterations.

TABLE 12. Summary of literature data on the carcinogenic and tumorigenic potentials of TCDD in animals

Animal	Number Used	Route of Administration	Response	Dose	Reference
Mouse	50 M[a] per group Swiss H/Riop strain	Gastric intubation weekly for 12 months starting with 10 week old animals	No effect in 5 animals examined 2 months after treatment ended	0.007 μg/kg[b]	135
			No effect in 5 animals examined 2 months after treatment ended	0.07 μg/kg[b]	135
			No tumors in 19 animals examined at end of treatment	7 μg/kg[b]	135
Rat	10 groups of 10 M	In diet for 78 weeks	All died in 2 to 4 weeks	50, 500 or 1000 μg/kg diet	136
			All died in 30 to 90 weeks	1 and 5 μg/kg diet	136
			50% dead at 95th week	500 ng/kg diet	136
		38% tumors	40% dead at 95th week	50 ng/kg diet	136
			40% dead at 95th week	5 ng/kg diet	
			20% dead at 95th week	1 ng/kg	136
		No tumors	60% dead at 95th week	Controls	136

Table 12 continued

	50 animals per group M/F[c]	In diet for 2 years	No effect Liver - 540 ng/TCDD/kg[e] Fat - 540 ng TCDD/kg[e]	.001 µg/kg[d]	78
			Increased urinary excretion of porphyrins in females Liver - nodules Lung - hyperplasia Liver - 5,100 ng TCDD/kg[e] Fat - 1,700 ng TCDD/kg[e]	0.01 µg/kg[d]	78
			Increased incidence of hepatocellular and squamous cell carcinomas Reduced evidence of Pituitary, uterine, mammary pancreas and adrenal tumors Liver - 24,000 ng TCDD/kg[e] Fat - 8,100 ng TCDD/kg[e]	0.1 µg/kg[d]	78
Monkey _Macaca mulatta_	8 F	In diet for 9 months	Anemia within 6 months Pancytopenia after 9 months Marked thrombocytopenia Tissue hemorrhages 5 of 8 died between 7 and 12 months Epithelial tissue changes	500 ng/kg diet[f]	2

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[a] M - Male

[b] Preliminary report remaining animals to be kept for life span study and observation for tumor development

[c] F - Female

[d] This is the dose supplied to each animal via the diet:

0.001 µg TCDD/kg body weight = 22 ng TCDD/kg diet
0.01 µg TCDD/kg body weight = 210 ng TCDD/kg diet
0.1 µg TCDD/kg body weight = 2200 ng TCDD/kg diet

[e] Terminal samples of liver and fat indicating accumulated levels of TCDD/kg of tissue after two years of treatment at the respective dosage levels

[f] Total exposure, 2-3 µg/kg body weight

F. Mutagenic and Cytogenetic Potentials of TCDD

Again, as with 2,4-D and 2,4,5-T, most of the mutagenic studies involving TCDD have been conducted in bacterial cultures or in plant and animal tissue cultures. Khera and Ruddick (75), however, have conducted dominant lethal tests in which male Wistar rats received TCDD, orally, at dosages of 4, 8 or 12 µg/kg per day for seven days. TCDD did not induce dominant lethal mutations during or in the 35 days following treatment. This 35 day period corresponded to the postmeiotic stages of spermatogenesis.

Green and Moreland (52) conducted a short-term investigation of several dioxins, using male Osborne-Mendel rats, to determine what potential these substances had to cytogenetic damage in rat bone marrow. In one study, all of the dioxins were tested via gavage in the rats for five consecutive days at 10 µg/kg per day. A second study involved TCDD being given separately by two routes. A single oral dose of 20 µg/kg TCDD or oral doses of 10 µg/kg TCDD for five consecutive days, and in other rats, single intraperitoneal doses of 5, 10 or 15 µg/kg TCDD were given. No evidence was found that any of the substances tested produced cytogenetic damage in the bone marrow of male rats under the conditions of the experiment. However, when rats of both sexes were treated twice weekly with TCDD at a dosage level of 4 µg/kg for 13 weeks, a significant increase in the number of chromosome aberrations was found by Green (51).

Hussain et al (65) evaluated the mutagenic activity of TCDD (99 percent pure) of three different microbial test systems. In the first study, TCDD significantly increased the incidence of reverse mutations in *Escherchia coli* Sd-4 when 2 µg/ml TCDD caused the bacteria to change from streptomycin dependence to streptomycin independence. This dosage was the only dose at which mutations were clearly observed.

In a second study, Hussain et al (65) examined reverse mutation from histidine dependence to histidine independence in *Salmonella typhimurium* strains TA1530 and TA1532. TCDD caused positive changes in TA1532 strain but negative results were seen in TA1530 strain which indicated that TCDD may act as a frameshift mutagen in this bacterial strain.

In a third study conducted by Hussain et al (65) slight prophage induction in *Escherichia coli* K-39 was observed. However, in this study the solvent DMSO was used which itself causes cellular effects.

Seiler (124) using plate assays to study the mutagenicity of TCDD found a positive response in *Salmonella typhimurium* strain TA1532, doubtful responses in strains TA1531 and TA1534, and negative responses in strains G46 and TA1530. Metabolic activation systems were not included in any of these microbiological assays.

Beatty et al (7) conducted a study with *in vitro* cultures of the mammalian cell types Hela, Balb-3T3, virus (SV-40) transformed 3T3 mouse fibroblasts, human foreskin fibroblasts and human lymphocytes. In all cases TCDD added in a final theoretical concentration of 10^{-6} to the culture medium prior to the addition of cells resulted in no significant inhibition of growth measured after a period of four days. Electron microscopic examination of the TCDD-treated cells did not reveal any changes in morphology as compared to untreated cells. Incubation of human fibroblasts and SV-101 cells with ^{14}C-labelled TCDD showed that incorporation of the TCDD into the cells did occur.

Kondorosi et al (79) found that TCDD did not impair the transfectivity of Qβ-RNA, thus confirming the assumption that TCDD did not react chemically with nucleic acid. Whatever mutagenic property it had must have occurred by the forming of a physical complex by "intercalation" in DNA, leading to frameshift mutation.

In summarizing the limited literature dealing with the mutagenic and cytogenic potentials of TCDD in animals, it was noted that daily oral doses of 4, 8, or 12 μg/kg TCDD given to rats for seven days did not induce dominant lethal mutations. Five daily oral doses of 10 μg/kg TCDD and a single oral dose of 20 μg/kg TCDD did not produce cytogenetic damage in bone marrow cells of male rats. Chromosome aberrations were detected when male and female rats were dosed twice weekly at 4 μg/kg TCDD for 13 weeks. Using microbiological systems, TCDD has been shown to induce mutagenic changes in some strains of bacteria.

V. SUMMARY OF THE LITERATURE REVIEW OF THE TOXICITY OF 2,4-D, 2,4,5-T AND TCDD IN ANIMALS

In summarizing the literature on the toxicity of 2,4-D, 2,4,5-T and TCDD in animals, the following general statements provided a concept of the overall toxicity of each compound as they related to each other and the effects they produced in experimental animal studies. Where possible, inclusive statements were given rather than individual species responses.

A. 2,4-D

1. The LD_{50} for single oral doses of 2,4-D in animals ranged from 100-2,000 mg/kg with the majority of LD_{50} values in the 300-800 mg/kg range.

2. Signs of chronic 2,4-D toxicity did not differ greatly from those seen in acute toxicity. No effect levels, seen when 2,4-D was given in repeated oral doses, ranged from 30 to 75 mg/kg.

3. Being a strong acid, 2,4-D was rapidly eliminated from the body mainly via the urine. The plasma half-life of a single oral dose was in the 3-12 h range. After high doses or repeated lower doses, 2,4-D accumulated in the tissues; with residue levels rapidly declining as evidenced by a half-life of 1 to 2 weeks.

4. No teratogenic signs were seen in rats fed repeated doses of 1,250 to 1,500 mg/kg 2,4-D of diet, nor when repeated daily doses of 8.75 mg/kg were given. Embryo toxic and fetotoxic responses appeared in rats and hamsters at repeated daily oral doses of 100 to 150 mg/kg.

5. Tumors were not produced in mice fed 46.4 to 100 mg/kg 2,4-D of diet nor in rats fed 1,250 mg/kg 2,4-D of diet for 18 to 24 months. Single subcutaneous injections of 21.5 to 215 mg/kg 2,4-D did not produce carcinogenic or tumorigenic responses in mice.

6. The 2,4-D was not highly cytotoxic in laboratory animals and did not cause increased mutation rates nor did it stimulate a mutagenic response in rats and mice. No mutagenic or cytogenic responses were seen in several studies using microbial systems for the detection of such toxicity.

B. 2,4,5-T

1. The acute toxicity for 2,4,5-T was in the same general range as for 2,4-D in most animal species. The LD_{50} values for single oral doses of 2,4,5-T ranged from 380 to 940 mg/kg in small laboratory animals.

2. Chronic toxicity studies in mice using repeated oral doses of 30-120 mg/kg 2,4,5-T produced no effect. An overlapping of adverse effects were seen, however, in doses of 60-140 mg/kg 2,4,5-T, depending on the strain of mouse studied. The no effect level for rats orally administered repeated doses of 2,4,5-T was approximately 30 mg/kg while as much as 300 mg/kg of diet could be fed with no adverse effects being noted. Threshold toxicity levels for adverse effects of repeated oral doses of 2,4,5-T in rats was approximately 100 mg/kg or 1,000 mg/kg of diet.

3. Single doses of 2,4,5-T were eliminated in animals, primarily unchanged, via the urine and feces over a period of a few hours up to about 7 days.

4. It was evident that 2,4,5-T induced embryotoxic and teratogenic responses in some strains of mice, rats and in hamsters when repeated oral doses of 20 to 400 mg/kg were administered. However, doses of 20 to 150 mg/kg in the same laboratory animal species produced a negative or no effect response. This indicated a great species and strain variation in response to 2,4,5-T as well as the fact that the embryotoxic and teratogenic potential of 2,4,5-T varied with the concentration of TCDD present. Levels of TCDD greater than 1 mg/kg were required to enhance the embryotoxic and teratogenic potential of 2,4,5-T. Embryotoxicity and teratogenic studies in pregnant rabbits, sheep and rhesus monkeys have been negative.

5. In most strains of mice, oral doses of 21.5 mg/kg or repeated doses of 60 to 100 mg/kg in the diet or drinking water and single subcutaneous doses of 2.5 mg/kg of 2,4,5-T did not induce tumor formation.

6. In animals, 2,4,5-T, like 2,4-D was not highly cytotoxic and did not increase mutation rates nor stimulate a mutagenic response in rats and mice. It produced, however, chromatid abnormalities in cultured human lymphocytes and affected the chromosomes and reproductive mechanisms in mouse and hamster bone marrow cells. These effects may have been due to cellular toxicity rather than genetic alterations. No mutagenic responses were seen in several studies using microbial systems for the detection of such toxicity.

C. TCDD

1. TCDD was an extremely toxic material with a single oral dose LD_{50} range of 0.6 µg/kg in male guinea pigs to 115 µg/kg in rabbits.

2. Chronic toxicity was manifested by hepatic necrosis, thymic atrophy and depletion of lymphoid organs. In mice and rats, repeated oral doses of 0.001 to 10 µg/kg for four to 13 weeks produced a no effect response for weight gain and no signs of toxicity were noted. Repeated oral doses as low as 1 µg/kg caused guinea pigs to become moribund and a repeated dose of 0.04 µg/kg decreased lymphocyte counts. Acne of increasing severity was produced in rabbits when doses of 0.04 to 400 µg/kg were applied repeatedly to the internal surface of the ear. A total oral dose of 2-3 µg/kg over a nine month period produced severe hematological changes and death in rhesus monkeys.

3. The primary route of excretion for TCDD in animals appeared to be the feces, with urinary excretion occurring at a much reduced rate. Liver and fat accumulated about 10 times higher levels of TCDD than did other body tissues. The half-life for TCDD in rats, following repeated exposure, was 12-15 days after termination of treatment.

4. It was apparent that TCDD caused birth defects and embryo mortality. Repeated daily oral doses of 0.1 to 2 µg/kg TCDD in pregnant mice produced no effects on the embryos; however, 3 µg/kg was the threshold level for production of cleft palate and kidney abnormalities. Single or repeated oral doses of 6.5 to 40 µg/kg TCDD were required to produce cleft palate in 50 percent or more of some strains of mouse embryos. Daily subcutaneous injections of 1 to 3 µg/kg TCDD produced cleft palate and kidney abnormalities in 50 percent or more of three different strains of mouse embryos. Repeated daily oral doses of 0.03 to 0.125 µg/kg TCDD produced no effect in some rat strains while doses of 0.125 to 2 µg/kg TCDD depressed fetal weight, lowered fetal survival and caused internal hemorrhages in fetuses. When teratogenic lesions appeared in rats, kidney abnormalities were more common than cleft palate.

5. No tumors were produced in a preliminary study where 0.007, 0.07, or 7 µg/kg TCDD was administered to mice in weekly oral doses for 12 months. When levels of 1 and 5 µg/kg TCDD of diet and 1, 5, 50 and

500 ng/kg TCDD of diet were fed to rats for 78 weeks, an overall tumor incidence of 38 percent was present in the test animals. No effects were produced when 0.001 μg/kg TCDD was given to rats via the diet for 2 years. A level of 0.01 μg/kg TCDD given via the diet for 2 years produced liver nodules and hyperplasia of the lung epithelium. A level of 0.1 μg/kg TCDD in the rats' diet for 2 years produced an increase in liver and lung carcinomas. Monkeys fed 500 ng/kg TCDD of diet for 9 months did not develop tumor but died of marked hematological alterations.

6. Daily oral doses of 4, 8 or 12 μg/kg TCDD given to rats for seven days did not induce dominant lethal mutations. Five daily oral doses of 10 μg/kg TCDD and a single oral dose of 20 μg/kg TCDD did not produce cytogenic damage to bone marrow cells of male rats. Chromosome abnormalities were noted in male and female rats dosed twice weekly at 4 μg/kg TCDD for 13 weeks. Using microbiological systems, TCDD has been shown to induce mutagenic changes in some strains of bacteria.

CHAPTER IV
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CHAPTER V

2,4,5-T/TCDD EPISODES

I. INTRODUCTION

The current controversy over the potential adverse human effects of 2,4,5-T and TCDD stem from a chain of events that occurred in the 1960s. The presence of TCDD as a contaminant, potent acnegen and acute toxin in the production of 2,4,5-trichlorophenol was documented in 1957 by Kimmig and Schulz (58). However, it was not until 1964 that concern over the levels of TCDD in 2,4,5-T herbicide was reported. In that year, the Dow Chemical Company experienced contamination problems during its expansion in production of 2,4,5-T to meet the requirements for Herbicide Orange by the U.S. military. They closed their production facilities and made extensive modification in the reaction conditions for the synthesis of trichlorophenol. By late 1965, the new technology developed by Dow Chemical Company permitted production of 2,4,5-T containing no more than 1 ppm TCDD (36).

Simultaneously, in 1964, the National Cancer Institute contracted for a screening study of a number of pesticides to determine if they were tumorigenic, teratogenic or mutagenic. Among the pesticides evaluated was 2,4,5-T herbicide. By 1967-68, preliminary data on 2,4,5-T from this screening study indicated that 2,4,5-T was teratogenic (15). The data were apparently provided to the press prior to actual publication. [When the manuscript eventually appeared in the scientific literature, in 1970, it contained a footnote indicating that the original sample of 2,4,5-T used in the screening tests contained approximately 30 ppm TCDD (23).] The press releases in the U.S. on the teratogenicity of 2,4,5-T were occurring in the same time period that South Vietnamese newspapers were publishing reports of an alleged increased occurrence of birth defects in areas sprayed with Herbicide Orange. These releases elicited far-reaching reactions from governmental agencies, segments of the scientific community and various lay groups concerned with environmental problems (2). On October 29, 1969, the President's Scientific Advisor announced that a series of coordinated actions was being taken by several governmental agencies to restrict the use of 2,4,5-T herbicide.

Additional animal experiments performed early in 1970 confirmed that pregnant mice did deliver some malformed offspring. The question then was one of whether, or to what extent, such animal data could be extrapolated to man. On April 14, 1970, the Secretary of Health, Education and Welfare (HEW) advised the Secretary of Agriculture that: "In spite of these uncertainties, the Surgeon General feels that a prudent course of action must be based on the decision that exposure to this herbicide may present an imminent hazard to women of child-bearing age." Accordingly, on the following day, the Secretaries of Agriculture; HEW, and Interior jointly announced the suspension of 2,4,5-T for "all uses around the home, recreation areas, and similar sites" and "all uses on crops intended for human consumption." Immediately thereafter, the Department of Defense suspended the use of Herbicide Orange in South Vietnam (7).

Numerous incidents involving suspected 2,4,5-T/TCDD poisoning of humans or livestock have been reported since this initial controversy. The most recent alleged episode involved veterans of the Vietnam Conflict. In March 1978, WBBM, a CBS-owned television affiliate in Chicago, Illinois, aired a special report on "Agent Orange: Vietnam's Deadly Fog". In the film, a number of past episodes allegedly involving 2,4,5-T and TCDD were examined. This chapter will review the available scientific data on these and other episodes, including industrial episodes, assessments in South Vietnam and the incident that occurred in Seveso, Italy, in July 1976. The medical data on many of these episodes are reviewed in Chapter VI.

The expression of units of weight, area, or volume has not been standardized between the various publications cited in this Chapter.

II. INDUSTRIAL EXPERIENCES

A. Industrial Processes

The herbicide, 2,4,5-T, was first commercially produced in the United States in 1944 (79). The quantity of 2,4,5-T produced and used in the United States and in world agriculture increased steadily until 1968-69, after which a sharp decline in its use occurred. Table 1 shows total U.S. Production data for 2,4,5-T, and how it was subsequently used, during the period 1961 through 1969. Approximately 34 percent (53 million pounds) of the total U.S. production was procured by the Department of Defense for use in South Vietnam. However, 8.9 million pounds of the 53 million pounds were not sprayed in South Vietnam, but rather destroyed by at-sea incineration in 1977 (see Chapter II). During the same period, 1961 through 1969, 50.6 percent (78.1 million lb) of the total U.S. 2,4,5-T production was used in domestic herbaceous and woody plant control programs.

The synthesis scheme for the industrial production of 2,4,5-T herbicide is shown in Figure 1. Forth (40) has described two different processes for the manufacture of the herbicide. The "Dow" process is a pressureless, high temperature process (>160°C but <200°C) requiring the alkaline hydrolysis of 1,2,4,5-tetrachlorobenzene to sodium trichlorophenate in the presence of ethylene glycol (an alcohol) and caustic soda (e.g., sodium hydroxide).

The second process, the "Boehringer" process uses high pressure (19.5 atmospheres) but low temperature (157°C) conditions in the presence of methanol, caustic soda, and 1,2,4,5-tetrachlorobenzene. Both processes will result in the formation of sodium trichlorophenate. The sodium trichlorophenate can be acidified to form trichlorophenol or may be used directly in the production of 2,4,5-T by adding chloroacetic acid. The production of the n-butyl ester (NBE) of 2,4,5-T is accomplished by

TABLE 1. Total United States production and use of 2,4,5-T herbicide for the period 1961 through 1969.[a]

Use	Million Pounds	Percent of Total
Herbicides Green, Pink and Purple[b]	1.6	1.04
Herbicide Orange[c]	51.4	33.27
Exports	23.4	15.15
Domestic Use	78.1	50.55
Total	154.5	100.01

[a] Total production and export data were from The Pesticide Review, 1970 and earlier issues, U.S. Department of Agriculture, Agricultural Stabilization and Conservation Service, U.S. Government Printing Office, Washington D.C. Data expressed in acid equivalents.

[b] Data based on estimated number of gallons of Herbicides Green, Pink and Purple used in South Vietnam, 1962-1964.

[c] Data based on estimated number of gallons of Herbicide Orange used in South Vietnam (10,645,904 gallons) plus the surplus 2,215,125 gallons remaining after termination of Operation RANCH HAND.

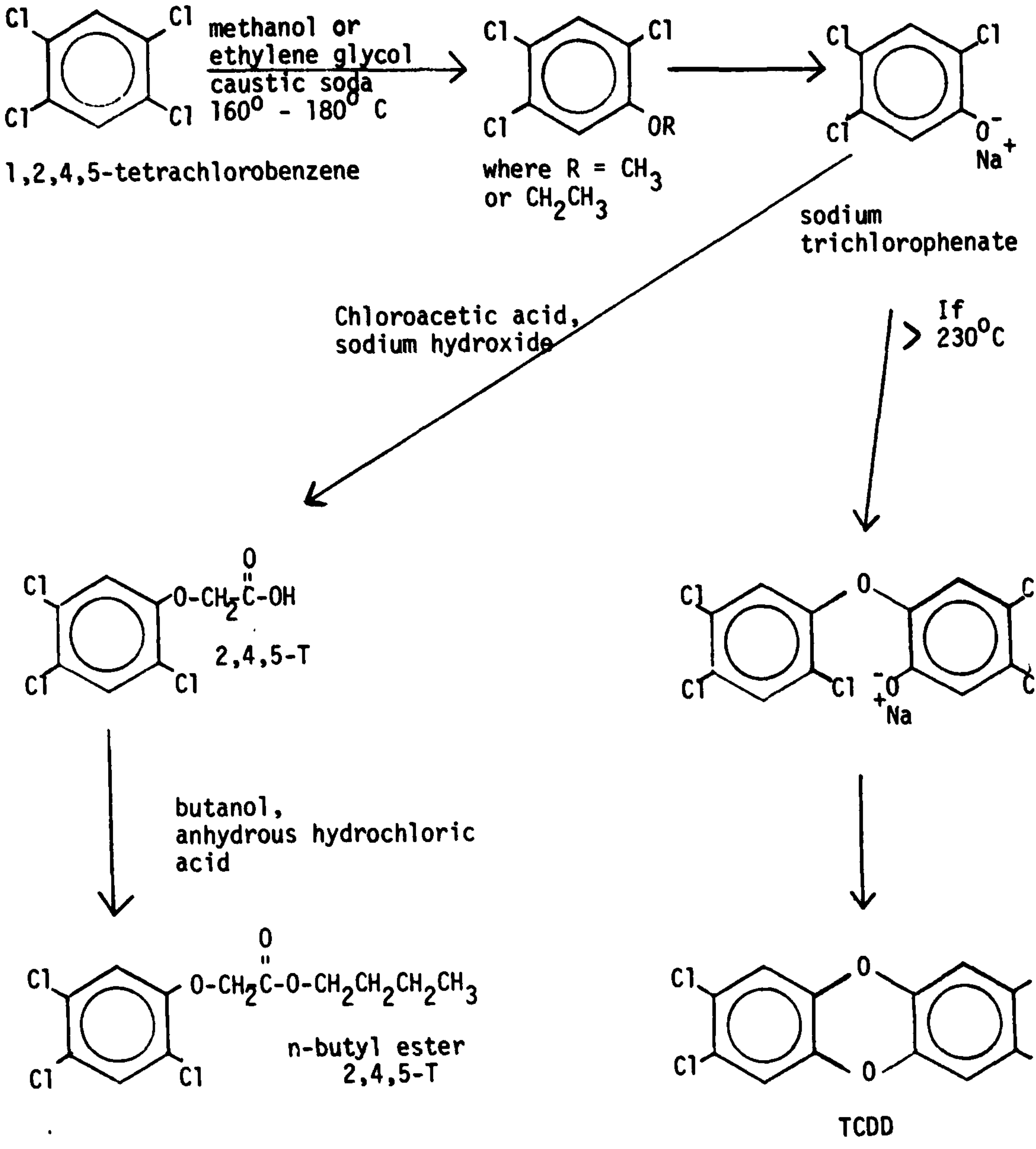
Cl
Cl
Cl
Cl
methanol or
ethylene glycol
caustic soda
160° - 180° C
1,2,4,5-tetrachlorobenzene
Cl
Cl
Cl
OR
where R = CH3
or CH2CH3
Cl
Cl
Cl
O-
Na+
sodium
trichlorophenate
Chloroacetic acid,
sodium hydroxide
If
> 230°C
O
O-CH2-C-OH
2,4,5-T
O
-O
+Na
butanol,
anhydrous hydrochloric
acid
O
O-CH2C-O-CH2CH2CH2CH3
n-butyl ester
2,4,5-T
O
O
TCDD

FIGURE 1. Synthesis scheme for production of the n-butyl ester 2,4,5-T (NBE 2,4,5-T) and site where formation of TCDD may occur.

esterification using butanol and anhydrous hydrochloric acid. TCDD is formed only during the formation of the phenol. Dimerization of the sodium trichlorophenate to form TCDD will occur in the reaction vessel during the alkaline hydrolysis of 1,2,4,5-tetrochlorobenzene. Maintaining low temperatures, ~160°-180°C, will minimize the formation of TCDD.

The reaction temperatures during the "Dow" process may become difficult to maintain. If the temperature of the hydrolysate rises above the normal 180°C, an exothermic reaction occurs after any residual solvent, e.g., glycol, is removed by distillation. This reaction, attributed to the decomposition of sodium-2-hydroxethoxide, starts at a temperature of 230°C and continues to 410°C. The heat generated by this reaction assists in the formation of TCDD through the dimerization of two molecules of sodium trichlorophenate. The rapid temperature increase in the reaction vessel, results in a pressure increase; failure to release the pressure has resulted in some of the industrial accidents that have been reported (46).

B. Industrial Episodes

In the years since the first commercial production of 2,4,5-T herbicide (1946-47), there have been numerous industrial episodes involving exposure to TCDD (and/or other chlorinated dibenzo-*p*-dioxins). The exposure to TCDD normally occurred during the handling of contaminated intermediate products (e.g., trichlorophenol, TCP). Fifteen of 23 episodes recorded in the literature were apparently associated with this "occupational" exposure. However, on eight occasions, explosions occurred, generally during the production of sodium trichlorophenate, and personnel were exposed to TCDD at the time of the accident, during the clean-up of the accident or from subsequent contamination of the workshop environment.

The first reported industrial accident occurred in Nitro, West Virginia, in 1949 (51). A total of 228 people were poisoned by the reactor residue during and/or immediately after the accident. No measures were taken to decontaminate the factories or to control the residue from the reaction vessel. In 1953, an accident occurred at a TCP factory in Ludwigshafen, Federal Republic of Germany (43). The 55 workers that showed chloracne and other acute toxic effects were exposed to the residue of the reaction vessel either during the accident or in the subsequent clean-up work. By 1957, Kimmig and Schulz (55) implicated TCDD as the causative agent of at least the chloracne seen in these workers.

The most thoroughly documented episode of occupational exposure has been by Jirasek et al (54,55) and occurred in Czechoslovakia between 1965 and 1969. In 1965, two technicians developed chloracne during the evaluation of a new production process for the manufacture of 2,4,5-T. At the time, it was assumed that they were exposed because of careless work and defective equipment under the pilot plant conditions. During the following two years, after the plant became operational, an additional

76 people developed chloracne. An investigation and study of the entire problem finally resulted in a shutdown of the operation and abandonment of the production in 1968. Jirasek et al have carefully described the afflicted individuals and have continued to monitor their health since the onset of the disease.

The above two episodes and other episodes involving occupational chloracne associated with the manufacture of chlorinated phenols are presented in Table 2. The clinical features of the affected cases described in the 23 episodes listed in Table 2 are described in Table 3. Note that most features observed were inconsistent with the exception of chloracne. Certainly, extended exposure to the major chemicals [TCP, pentachlorophenol (PCP), 2,4-D or 2,4,5-T] must have complicated the observed clinical features. In addition to TCDD, it should also be noted that TCP may also contain significant concentrations of hexachlorodibenzo-*p*-dioxin, while PCP may contain hexa-, hepta- and octachlorodibenzo-*p*-dioxin.

An extensive review of occupational chloracne has been prepared by Crow (24) and Kimbrough (56). With one exception, men have been almost exclusively affected by the disease due to the occupational position they have historically maintained in the factories producing the chlorinated phenols. The few women and children that have been afflicted were exposed because of contact with clothing worn by the men. Goldmann (43) reported that a female animal nurse developed chloracne by contact with contaminated test animals. The one industrial incident where a large number of women were afflicted with chloracne was reported by Braun (17) in 1959. Braun examined 114 women and 9 men, who in the course of a year became afflicted with chloracne in a condensor factory where chloronaphthalenes of different degrees of chlorination were used as dip-waxes. The reporting of this incident is important because of contradictory statements in the literature on the differential sensitivity of different people (including sexes) to chloracne. From the examination of the women Braun concluded the following:

1. Age was of no importance within the range of 20 to 45 years.

2. Strongly adiposed persons were more likely to be afflicted.

3. Seborrhoic types with greasy skin and open pores and scars of previous *Acne vulgaris* were sooner and more severely afflicted.

4. Non-affliction was definitely extremely rare under the circumstances. Only four women (2 of them sisters) with a very smooth and fine skin through which the veins showed bluish when they were at rest remained free from the alterations due to the disease.

5. The occurrence of chloracne had no real relationship to hair color and skin pigmentation.

TABLE 2. Industrial incidents associated with the manufacture of chlorinated phenols.

Year	Country	Manufacturer/Location[a]	Production Product[b]	Primary Source of Exposure	Number of Cases	Years from Incident to Last Observation[c]	Reference
1949	United States	Monsanto/ Nitro, West Virginia	TCP	Explosion	228	4	51, 73
1949	West Germany	———/ Nordrhein, Westfalen	PCP, TCP	Occupational	17	1	11
1952	West Germany	———/———	TCP	Occupational	60	-	12
1952-53	West Germany	Boehringer/———	TCP	Occupational	37	-	46
1953	West Germany	BSAF/ Ludwigshafen	TCP	Explosion	55	24	51, 43
1954	West Germany	Boehringer, Ingleheim/ Hamburg	TCP, 2,4,5-T	Occupational	31	9	58, 12
1956	France	Rhone Poulenc/Grenoble	TCP	Explosion	17	2	30
1956	United States	Diamond Alkalai/ Newark, New Jersey	2,4-D, 2,4,5-T	Occupational	29	13	16, 68
1956	United States	Hooker/———	TCP	Occupational (?)	-	-	46
1960	United States	Diamond Shamrock/———	TCP	Occupational (?)	-	-	46

Table 2 (continued)

1962	Italy	———/———	TCP	Explosion	5	-	47, 51
1963	Netherlands	Philips-Duphar/ Amsterdam	TCP	Explosion	50	14	14, 26 51
1964	USSR	———/———	2,4,5-T	Occupational	128	-	50, 74
1964	United States	Dow Chemical/ Midland, Michigan	2,4,5-T	Occupational	60	6	38
1965-69	Czechoslovakia	Spolana/———	TCP	Occupational	78	6	54, 55 67
1966	France	Rhone Poulenc/ Grenoble	TCP	Explosion	21	-	46
1968	United Kingdom	Coalite and Chemicals Products/ Bolsover, Derbyshire	TCP	Explosion	79	9	51, 60
1970	Japan	———/———	PCP, 2,4,5-T	Occupational	25	3	64
1972	USSR	———/———	TCP	Occupational	1	1	81
1973	Austria	Linz Nitrogen Works/———	2,4,5-T	Occupational	50	-	40, 46
1974	West Germany	Bayer/Uerdingen	2,4,5-T	Occupational	5	-	40, 46
1975	United States	Thompson-Hayward/ Kansas City, Kansas	TCP	Occupational	-	-	46

Table 2 (continued)

1976	Italy	ICMESA/Meda	TCP	Explosion	134	2	69, 80

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[a] The name of the factory or company and its location was cited whenever it was available because considerable confusion exists in the published literature as to what incident is addressed. The absence of data indicates the information was not available.

[b] TCP = trichlorophenol, PCP = pentachlorophenol

[c] Frequently individuals involved in an incident, and who were examined initially, may have also been examined at a later date. The years that lapsed from the exposure until the most recent examination are cited in this column. The absence of a number indicates that only an initial examination was reported in the referenced literature.

TABLE 3. Some clinical features observed in cases of chloracne associated with production of 2,4,5-T and other chlorinated phenols.

Frequency observed	Clinical Features	Additional Notes
Consistent	Chloracne	In worst cases, chest and inguinal area affected and scarring generally increased.
Occasional	Prophyria cutanea tarda	Increased excretion of urinary uroporphyrin or coporporphyrin or both.
Inconsistent	Hyperpigmentation of the skin	Usually prominent on face and consisted of grayish or brownish tone to the complexion.
	Hirsutism	Noticeable between the outer edge of the eyebrow and the temple hair margin.
	Enlarged, tender liver	
	Excessive mechanical fragility of the skin	
	Neuromuscular symptoms	Severe pains in the chest and pain and weakness in extremities

Table 3 (Continued)

Mucous membrane irritation	Itching of the eyes and frequent tearing, hyperemia of the nasal mucosa, and inflammation of the buccal mucosa.
Irritability	Nervousness and insomnia.

6. A simultaneous psoriasis or a systemic eczema in the previous case history, or a pregnancy, did not make any difference and had no perceptible effect on the course of the chloracne. One woman reported that she had noticed a worsening of her chloracne after her delivery.

7. During the menstrual period, the pimples on the cheeks of some of the women were temporarily more prominent.

8. Dirty and untidy women took ill sooner and more severely than those who placed great importance on cleanliness and hygiene.

The persistence of dioxins in the environment of an industrial plant has been documented by Jensen (53). He reported on two cases of chloracne in employees of an outside contractor that had been working on a piece of equipment exposed (but thought to have been decontaminated) to TCDD three years earlier in an industrial explosion in Derbyshire, England in 1968. A young son of one of these employees also develeoped chloracne. The presumed source of the child's contamination was the father's working clothes.

An episode involving the deliberate synthesis of TCDD has been reported by Oliver (65). This episode involved three young (male) scientists working with pure TCDD in the laboratory. Two of the men were exposed to the dioxin while attempting to synthesize it by heating trichlorophenol in an alkaline solution in the presence of a catalyst or by heating prepared potassium trichlorophenate in a closed system. Both men wore overalls and plastic gloves and allegedly took the utmost care to avoid inhalation or skin contact. The third man was a colleague of the other men and had been working with the diluted dioxin standards they had prepared. His work also had been done with the utmost caution and with special care to avoid personal contamination. Three clinical features were common to the three men, namely, chloracne, hyperpigmentation and hypercholesterolemia (increased levels of cholesterol). None of the three patients had evidence of acquired porphyria. However, two patients developed hirsutism (excessive facial hair) two years after the exposure. These same two also reported that when the hirsutism developed, other symptoms occurred, e.g., loss of appetite, oppressive headaches, and an unusual loss of vigor and drive with excessive fatigue. Oliver concluded from the evidence that those accidentally exposed to dioxin (TCDD) may be subject to delayed toxic effects for at least two years.

III. VIETNAM EPISODE

As noted in Chapter I, approximately 53 million lb of 2,4,5-T were in the 13.2 million (gal) of Orange, Purple, Pink and Green procured by the Department of Defense. However, 8.9 million lb of 2,4,5-T were in the surplus Herbicide Orange. Thus, approximately 44 million pounds of 2,4,5-T were sprayed in South Vietnam from 1962 through 1970. As noted in Chapter I, an estimated 368 lb of TCDD were probably present in Herbicides Orange, Purple, Pink and Green.

Irish et al (52) have stated that among all the controversial subjects that were part of the conflict in Vietnam, the use of vegetation-control chemicals received an undue amount of publicity that was generally critical. They noted that the Department of Defense was not insensitive to the critics' pronouncements; justification for continuation of the RANCH HAND program had been periodically reviewed. The conclusions of all the evaluations prior to 1969 recognized that defoliation had reduced the incidence of ambushes, saved lives and disrupted enemy tactics. The issues of long-term ecological damage or potential adverse human health effects due to the herbicides were little discussed until the late 1960s. These issues when viewed in context with the realities of the military conflict were of minor concern, especially since the available scientific data did not support the justification for greater concern. It should be noted that in 1967 the Department of Defense had contracted with the Midwest Research Institute (MRI), Kansas City, Missouri, for an in-depth report on the assessment of ecological effects of extensive or repeated use of herbicides (49). Following its publication in December 1967, both the National Academy of Science (NAS) and the American Association for the Advancement of Science (AAAS) reviewed the document and concluded that MRI had "done a creditable job of assessing the scientific literature related to herbicides and their ecological effects." However, both organizations felt that the report represented "only a first step in investigating further the ecological effects of intensive use of herbicides" (3). Some of the conclusions that the MRI reported (49) included:

1. The greatest short-term or long-term direct ecological consequence of using herbicides in Vietnam or anywhere else is the destruction of vegetation. As long as soil sterilization is not an objective, destruction of vegetation by herbicides is a selective process, denuded earth does not occur especially in forest spraying. Furthermore, the end result of the use of herbicides from an ecological standpoint is that the ecosystem is set back to an earlier sere, i.e., an earlier stage of plant succession.

2. The long-term effects on wildlife may be beneficial or detrimental. Studies in other countries have shown that herbicidal treatment of forested areas improves wildlife habitat and is favorable to animal populations. The extent and pattern of herbicide treatment in Vietnam have no precedent; therefore, it is difficult to predict effects on wildlife with any accuracy.

3. The herbicides used in Vietnam will not persist at a phytotoxic (plant toxic) level in the soil for a long period of time. On the basis of the average temperatures and rainfalls in Vietnam, it would be reasonable to expect that the chlorophenoxy acid esters will be dissipated quickly.

4. The possibility of lethal toxicity to humans, domestic animals or wildlife by use of the herbicides used in Vietnam is highly unlikely and should not be a matter of deep concern.

5. Herbicides seldom persist in animal or insect tissues. Toxic transfer to the next higher animal in the food chain is minimal. In fact, biological concentration does not occur with most herbicides, since they are readily excreted from animals.

In September 1968, the U.S. Department of State released an assessment of the ecological consequences of the defoliation program in Vietnam. Tschirley (75), a plant ecologist and the author of the Department of State report, published his assessment in *Science* (the Journal of the AAAS) in February 1969. The major conclusions reached by Tschirley after his four-week visit to South Vietnam included:

1. The defoliation program has caused ecologic changes. These changes are not irreversible, but complete recovery may take a long time. Regeneration of the mangrove forest to its original condition is estimated to require about 20 years.

2. The effects of defoliation on animals is not known, but it does not appear to have been extreme. There is no evidence to suggest that the herbicide used in Vietnam will cause toxicity problems for man or animals.

In March 1969, the Society for Social Responsibility in Science, sponsored a five-week trip for two zoologists to Vietnam with the objective of supplementing Tschirley's observations. The subsequent report, written by Orians and Pfeiffer (66), was published in May 1970. Their conclusions included:

1. The ecological consequences of defoliation were severe, especially in areas receiving repetitive applications of defoliants.

2. Evidence was found of moderate to severe defoliation of trees and herbs in areas many miles removed from sites of application.

3. Little evidence of toxic effects of the herbicides to animals was found, although one report was received (through an interview) of many sick and dying birds and mammals in forests following defoliation. The report was not investigated.

4. No evidence was found that the herbicides had direct adverse effects on human health. The defoliation program however, has had tremendous psychological impact upon the Vietnamese people, and the crop destruction program may have impacted on the availability of food for women, children and elderly people in the highland regions of South Vietnam.

The first reports of human birth defects allegedly attributed to Herbicide Orange appeared in Vietnamese newspapers between June 26, 1969 and July 5, 1969 (2). The public and scientific furor caused by these reports resulted in two surveys of South Vietnamese hospital records conducted independently by Cutting et al (25) and Meselson et al (63). An evaluation of both documents in 1971 by an advisory Committee on 2,4,5-T to the Administrator of the Environmental Protection Agency (2) concluded with the following summary:

> Summarizing the Vietnam data on human embryotoxicity, it can be said that (1) the sample of births surveyed was from year to year a variable but usually very small fraction of the total number, (2) it was quite unrepresentative of the geographic and ethnic distributions, (3) the heavily sprayed and otherwise exposed areas were greatly underrepresented, and (4) the birth records were not trustworthy and, therefore, the rates of stillbirth, and especially of congenital malformation, derived from them were equally unrealiable. For example, the overall congenital malformation rate found in South Vietnam, 4.91 per 1000 livebirths, is about half of what was reported in other studies in various parts of Asia, and possibly a quarter of what might actually exist at term. A further indication that the newborn children were not carefully examined is the absence of Down's syndrome in the list of specific malformations compiled by the Army survey [Cutting et al (25)] despite the fact that some Oriental populations have been reported to have an incidence of this condition not unlike that in Western populations.
>
> Finally there is, and can be, no precise knowledge or reasonable approximation of the exposure to 2,4,5-T (and hence, TCDD) experienced by pregnant Vietnamese women, including what amounts they ingested or absorbed and when this may have occurred during pregnancy. Thus, any attempt to relate birth defects or stillbirths to herbicide exposure is predestined to failure. It can only be concluded that the birth records that have been surveyed, and probably any that will be surveyed in the future, for South Vietnam for the period 1960-1970 cannot answer positively the questions about possible adverse prenatal effects following human exposure to 2,4,5-T. It must be emphasized, however, that the searches that have been made almost certainly would have revealed any marked increase in the incidence of birth defects or the introduction of a striking defect such as that produced by thalidomide. In spite of considerable effort, no such occurrences were found.

Following the publication of the above two surveys, some additional reports of birth defects in South Vietnam were released. One of these was by Tung et al (77) of North Vietnam (Democratic Republic of Vietnam). They reported that out of a total of 903 South Vietnamese taking shelter in the North and grouped in hospitals and lodgings in Hanoi, 19 adult women, including 4 mothers, and 70 children between the ages of 6 and 14 had been directly hit by herbicidal sprays while living in South Vietnam. The report went on to state that of the above four mothers, two had given birth to children with Down's Syndrome (Trisomy 21). In addition, among the 70 children between the ages of 6 and 14, numerous cases of deformations were evident, e.g., ocular lesions, exaggerated lumps on the forehead, valgus feet (i.e., feet that are bent outward) and a high frequency of chromosomal aberrations in lymphocytes and leucocytes. Following a summary of their data, Tung et al (77) concluded by stating:

> Though still limited in number, our clinical observations confirm the results obtained on animals by American researchers. The massive and prolonged utilization of defoliants besides permanent ocular lesions, can cause chromosomic alterations among a population obliged to cling to ancestral soil and these alterations can provoke among their progeny congenital malformations the importance of which remains to be determined. In the abominable history of wars, have we ever seen such an inhuman fate reserved for the survivors except in the case of atomic war?

In reviewing the report by Tung et al (77), the Dow Chemical Company (8) noted that basically, the whole study was a result of a seemingly hit and miss clinical examination of some refugees from South Vietnam who had lived in regions where defoliants had been applied. There was no record of exposure except that most had been sprayed at one time or another with something. Dow further stated: "Trying to correlate cause and effect from the published data is completely frustrating and futile. There is no doubt that these authors saw some ill people, but to reach the conclusion that their problems were caused by 2,4,5-T rather than the ravages of war is speculation."

A study similar to Tung et al (77) was reported by Rose and Rose (71) in 1972. They interviewed 98 refugees in Hanoi who claimed to have been repeatedly sprayed with defoliants while in South Vietnam. Abortions were reported for humans and domestic animals and monstrous births were said to have occurred. Deaths evidently occurred among human, domestic animals, fish and fowl.

The charge by Tung et al that TCDD in 2,4,5-T was reponsible for much of the Down's Syndrome seen in South Vietnam was also made by Grumner as reported by Honoroff (48). Grumner, apparently of Rockstock University, German Democratic Republic, claimed to have observed high incidences of children with Down's Syndrome while on a trip through North and South Vietnam. Honoroff quoted Grumner:

> Provided that it be understood that this estimate cannot be anything but a cautious one, it may be assumed that there are at least 25,000 children with hereditary defects in South Vietnam. This does not include all the unborn babies whose mothers were sprayed during the missions that were flown in recent months. It does not include those who were stillborn, or died soon after birth, on account of their serious chromosomatic defects. Even

> after the war, it will probably be possible to arrive at only an approximation of the entire scale of this crime since one will only be able to examine the survivors when the time comes.

In 1973, Tung et al (78) reported an increase in the number of persons with primary liver cancer in proportion to all cancer patients admitted to Hanoi hospitals during the period 1962-1968 (790 liver cancer cases out of 7,911 cancer cases, 10 percent) as compared to the period 1955-1961 (159 liver cancer cases out of 5,492 total cancer cases, 2.9 percent), which was prior to the start of herbicide spraying. The authors attributed this increase to exposure as a result of the spraying of herbicides containing TCDD in South Vietnam during the 1960s [however, a recent IRAC monograph (50) noted that limitations in the reporting of the study make impossible an adequate assessment between the incidence of liver cancer and herbicide spraying in South Vietnam]. A further factor of importance has been suggested by Ford et al (39). They noted that at least in Thailand, consumption of aflatoxin-contaminated food was highly correlated with liver cancers. Aflatoxin is a naturally occurring contaminant of cereal crops.

In 1974, the National Academy of Science (NAS) (21) announced the results of studies conducted in South Vietnam in 1972 and 1973. The NAS Committee could find no conclusive evidence of association between exposure to herbicides and birth defects in humans. Available records of two major Saigon hospitals and evaluation of records in a third, as far as they went, showed no consistent pattern of association between rates of congenital malformations and annual amounts of herbicides sprayed. The Committee recognized, however, that the material was not adequate for definite conclusions. The Committee was also unable to confirm or deny reports that some humans (especially the Montagnards) and domestic animals became ill or died after exposure to herbicide sprays or after eating treated plants or drinking contaminated water. The Committee also attempted to assess the social, economic and psychological effects of the herbicide program. The impact of the program on the population "appeared relatively trivial as compared with other aspects of the upheaval in that country." Evidence was obtained that numbers of families moved away from their traditional homes because of the herbicide spray program but few were actually identified.

In a letter of transmittal for the NAS report (21), the President of NAS stated: "On balance, the untoward effects of the herbicide program on the health of the South Vietnamese people appear to have been smaller than one might have feared."

IV. EASTERN MISSOURI HORSE ARENA EPISODE

In August 1972, the Missouri Division of Health, St. Louis, Missouri, and The Center for Disease Control, Atlanta, Georgia (59) reported an investigation of a horse arena in eastern Missouri where 54 of 57 horses exposed to the arena had died of an illness characterized by skin lesions, severe weight loss and heptotoxicity. Birds, dogs, cats, insects and rodents were also found dead in and around the arena, and one 6-year-old girl exposed developed epistaxis, gastrointestinal complaints, and severe hemorrhagic cystitis (characterized by blood in the urine). Analysis of urine cultures for bacterial and viral pathogens was negative. Three other persons developed milder illnesses consisting primarily of transient headaches and nausea after exposure to the arena. The toxic substance(s) responsible for the illness was not at that time identified.

In the investigation of the illness, Lobes et al (59) found that the outbreak coincided with treatment of the arena floor for dust control with approximately 2,000 gal of salvaged motor oil. The treatment occurred on May 26, 1971. On May 30, the stable owners reported that "hundreds" of birds were found dead on the floor of the arena barn. Within the next few weeks, cats, dogs, rodents and horses began to die. The four people cited above [2 adults and 2 children (both girls)] had more than occasional exposure to the arena barn during the six months following the oil spraying. These individuals were first examined in mid-August 1971.

The report (59) also noted that similar horse illnesses and deaths occurred in two other horse arenas in the eastern Missouri area sprayed by the same salvage oil company. The three arenas had been sprayed within one month of each other. Subsequent to investigation, soil from all three arenas was excavated and disposed. No further problems occurred following the excavations.

In 1974, laboratory analysis of soil samples taken from the initial arena implicated 2,4,5-trichlorophenol (TCP) and TCDD as the probable toxic substances (29). The actual levels of TCDD in these soils however were not published until 1975, when Carter et al (19) provided more details on the exposure and the probable source of the TCDD in the salvage oil. The horse arena soil was found to contain 31.8 to 33 μg of TCDD per gram (ppm) of soil. In addition, further investigations revealed that the sludge used to spray all three arenas came from a common storage tank at the salvage oil company. It was suspected that TCDD and TCP were in distillate residues collected by the salvage company from a hexachlorophene producer in southwestern Missouri. Between February 1971 and October 1971 the salvage oil company obtained and stored 18,000 gal of the distillate in a storage tank from which the sludge for spraying the three arenas was obtained. In late 1971 the hexachlorophene plant and subsequently the salvage oil company both discontinued operations. The residue remaining in the tank originally used to store the distillate residue at the plant site was sampled in 1974. It contained TCDD in concentrations of 306 to 356 μg/g (19).

Case (20) has described some of the clinical studies performed on the horses involved in this episode. Kimbrough et al (57) has recently (1977) detailed the epidemiology and pathology associated with the poisoning episode.

Commoner and Scott (22) have reviewed the Missouri Horse Arena Episode in an attempt to provide consultative data to the Italian Government in the wake of the Seveso, Italy episode. Their review focused on the human reactions (symptoms) to accidental TCDD exposure and the problem of soil degradation of TCDD. They also provided an excellent chronological account of the episode.

Beale et al (13) have recently re-examined the young girl who had developed hemorrhagic cystitis following repeated exposure to TCDD in one of the horse arenas sprayed with the waste oil. In the 5-year interval since exposure, the patient had grown normally, and both her height and weight were above the 75th percentiles. Detailed physical, chemical and neurological examinations were also conducted and found to be normal. The same studies were done on the patient's sister and mother, exposed simultaneously, but less extensively to dioxin, and the results were also normal. Beale et al (13) concluded: "Our experience demonstrates that people exposed to dioxin can recover completely with no apparent sequela from the toxin. It remains to be determined whether the exposure to dioxin in these children will result in abnormal pregnancies or affect their offspring."

V. THE SEVESO, ITALY EPISODE

Perhaps the most publicized chemical accident in modern times is the TCDD episode in Seveso, Italy. This episode has attracted worldwide interest and concern. Hundreds of scientists, physicians and veterinarians have participated in either on-site inspections, conferences, or consultations into the various facets of this episode. Although the Seveso, Italy episode did not involve 2,4,5-T herbicide, it did involve the production of trichlorophenol. The trichlorophenol was in this case used in the production of hexachlorophene. Nevertheless, this episode represents to many people the inherent danger associated with the industrial production of 2,4,5-T.

Data on levels of TCDD found, the magnitude of the contamination and the extent of human and animal illness have just recently begun to appear in the scientific literature. The following scenario of the episode has been assembled from this literature.

The episode of TCDD poisoning occurred on 10 July 1976 in Seveso, Italy, a small town 40 kilometers (km) north of Milan (40,46). The source of the TCDD was a chemical factory that produced trichlorophenol through the alkaline hydrolysis of tetrachlorobenzene (see Figure 1). When the temperature in a steam-heated reaction vessel rapidly increased, a safety disk ruptured sending a plume of trichlorophenol, TCDD and other products 30 to 50 meters (m) high above the factory. The cloud apparently rose into the air, cooled and came down over a cone-shaped area about 2 km long and 700 m wide.

The chemical plant involved was the Givaudan ICMESA (Swiss-owned) chemical plant. At the time of the incident, there were some 2,000 kg of reagents and reaction products in the reactor (sodium trichlorophenate, soda, sodium chloride, ethylene glycol, tetrachlorobenzene and secondary reaction products) (9). Based on determinations made by production officials it was estimated that 4,000-500 kg of reaction product was discharged into the atmosphere (9,27). The amount of TCDD dispersed with the other reaction products has been estimated to have ranged from 650 grams to 1,700 grams (27,69). A sample of the escaped product taken from the reactor head for analysis revealed the presence of 3.5 percent TCDD (35,000 parts per million TCDD) (9).

The accident occurred on a Saturday. By the following Monday, a site inspection of the area revealed phytotoxic effects (brown discoloration and drop-like perforation of the leaves) for a distance of some 1,000-1,300 m in a triangle with a base of approximately 400 m and a vertex of 100 m centered on the factory (9). Several measurements of TCDD on vegetation in this area and areas adjacent to the factory were in the 1 to 15 ppm range, with one reading as high as 50 ppm (69).

Reggiani (69) reported that animals (birds, rabbits and chickens) were beginning to die 2-3 days after the accident. A few children and some adults who had been directly seized by airborne dust consisting of the reactor content were complaining of nausea and presenting skin lesions of various aspects and extension but mainly redness and swelling. Some of the children were hospitalized and the physicians in charge warned that beyond overt signs of injury pointing to the action of caustic material causing burns and blister formation, they also had to consider the possibility of a contact or ingestion of a still unknown quantity of TCDD.

In the meantime numerous Italian laboratories and the Givaudan Laboratories cooperated in mapping out the polluted zones, determining TCDD on soil, vegetation and buildings by gas chromatographic-mass spectrometric techniques (9,41,69). In addition, the Regional Veterinary Service assisted in drawing up the map, working from animal death patterns and TCDD levels in the liver of surviving animals. Highest TCDD levels were found in herbivorous animals (41).

About 1,000 assays led to the area being divided into two zones. Giovanardi (42) reported that the first zone, Zone A, was a triangular-shaped area covering approximately 1000 hectares (ha). This area, located south south-east of the ICMESA factory and downwind at the time of the accident, had estimated soil levels of TCDD greater than 0.001 ppm [Reggiani (69) later described this area as having TCDD levels greater than 10 ppb]. The 700 inhabitants of this area were evacuated in three stages, on 26 July, 28 July and 2 August 1976. In the second zone, Zone B, soil levels of TCDD were detectable but less than 0.001 ppm [Reggiani (69) defined the soil levels of TCDD as between 0.1 and 10 ppb]. This area covered approximately 250 ha and was divided between a large urban center and an extensive rural area with some small residential aggregates (42). This area had a population of 4,900 and was not evacuated. For the people in Zone B, recommendations were issued to reduce the possibilities of exposure in particular for the children and the women (69). A third zone, Zone C or "Respect Zone," covering a total of about 1,430 ha was also delineated. Occasional concentrations of less than 0.1 ppb TCDD in soil were found in this zone. The population of this area was approximately 40,000 people (69).

By late August 1976, an extensive surveillance system of the health of the population was established covering the acute and mid-term effects of the exposure as well as the long-term effects. General and special medical examinations, laboratory tests at given intervals, course and outcome of pregnancies, examinations of abortions, rate of stillbirths, followup of newborns, morbidity and mortality of the population, and a cancer registry were all set up to detect any abnormality of the health of the community for which an exposure to TCDD could be postulated. The medical health surveillance program was extended to 11 districts with a total population of 216,000 (9,14,37,69).

Periodically, reports of clinical damage to the population of Seveso have appeared in the press and scientific literature (38,40,41, 46,80). However, the most complete analyses of health data have been recently published by Reggiani (69). He concluded:

> The Seveso accident has not revealed up to now toxic effects in humans, which have not been observed in other episodes. Chloracne, the typical skin lesion, has occurred in children with tendency to spontaneous and rapid healing. The peripheral nervous system has perhaps been attacked and reacted with subclinical signs of impairment. Signs of involvement of the liver without apparent functional disorders have occurred. No other organs or functions have been impaired. There has been no derangement of the gestation, no foetal lethality and loss, no gross malformations, no growth retardation at term and no cytogenetic abnormalities. The immunocapability of the population, not even of the children with chloracne, has not been attained.

VI. GLOBE, ARIZONA EPISODE

Globe, Arizona was another site of possible human exposure to TCDD. In 1969, the U.S. Forest Service applied 3,680 lb of 2 (2,4,5-trichlorophenoxy) propionic acid (Silvex) and 120 lb 2,4,5-T in the Kellner Canyon-Russell Gulch spray project near Globe (76). The reports of harmful effects to animals and people from the spraying began during and immediately after the spray treatment. The complaints included damage to vegetation off the spray project area, deformed animals and human illnesses. Although the Forest Service investigated the allegations, many of the local citizens were dissatisfied with the reports and the case continued to fester until, in February 1970, it attained national attention. Television newscasts showed deformed animals alleged to have been caused by the herbicides.

On February 13, 1970, a public hearing was held in Globe. As *Time Magazine* (4) reported, the local veterinarian insisted that he had noticed nothing out of the ordinary in local animals. Doctors too were puzzled. Said one: "I keep trying to see the relationship between the spraying and the illnesses, but I have simply not found anything." *Time* (4) also reported that: "The investigators holding the public hearing ended up perplexed and incredulous. In a paranoid outburst, the investigators were accused of being impostors, really representatives of chemical manufacturers in clever disguise."

To look further into this episode, The Office of Science and Education, USDA, established an investigating team to assess the allegations against the Kellner Canyon-Russell Gulch Spray Project. Tschirley et al (76) published the results of the investigating team following on-site inspections of the spray project area, February 16-20, 1970. Tschirley et al, attempted to assess numerous parameters that would contribute to a comprehensive assessment of the episode. Some of these parameters included: (1) assessment of herbicide damage to plants off the project area, (2) effects of plant diseases, (3) effects of air pollution, (4) residue analyses of soils, plants and animal tissue, (5) observations of fish and wildlife, (6) evaluations of the health of domestic animals and (7) interviews with many of Globe's citizen and physicians. Some of the conclusions reached by Tschirley et al (76) were:

1. There was clear evidence of drift of herbicide outside the project area.

2. There was evidence of woody plant mortality from root rot, and also visible damage to certain yard trees from several kinds of birds and insects.

3. Reports from wildlife specialists indicated no significant effects on birds, deer and other wildlife.

4. With the exception of soil from the site where the herbicide was loaded aboard the helicopter, no residues of 2,4-D, 2,4,5-T, Silvex or TCDD were found in any of the substrates analyzed.

5. Information obtained from owners of livestock and observations of animals did not indicate any illnesses that do not commonly occur in other regions. No association was found between the herbicides and the deformed animals shown on the television newscasts.

6. Human illnesses had been reported by several residents in the Globe region. Many of the residents with complaints were interviewed

by a medical member of the panel. The complaints were those that commonly occurred in the normal population; no cases of chloracne were reported. One individual had an eye irritation from steam cleaning an empty herbicide drum. Nine doctors serving the area of Globe were interviewed and there was general agreement that there had been no significant increase in human illness related to the spraying.

Tschirley et al (76) summarized their panel report by stating: "Significant in evaluating the Globe situation was the emotional peak of its inhabitants. The complaints offered were those occurring in normal populations, with many of them (especially in the adults) being quite subjective. With the exception of the skin rash and eye irritation experience by one subject, it is highly unlikely that the ailments described were related directly to the spraying. However, the psychosomatic effect of an aroused public very likely has played a role. It is also important to note that except for three subjects all of the complaints dated only from the June 1969 spraying, despite the Forest Service having sprayed the same area three other years."

A subsequent report was published by Roan and Morgan (70) of analytical results of selected human tissue collected by Tschirley et al (76) and of an epidemiologic study of the hospital records. Roan and Morgan concluded:

> We cannot find any evidence that there was long-term exposure of residents of the Globe, Arizona area to chlorophenoxy herbicides, or significant contamination of water supplies in this area with 2,4-D, 2,4,5-T, Silvex or metabolites of these herbicides. Nor have we found contaminants such as TCDD that may be associated with one or more of the above technical grade products. Statistics on reproductive mortality and morbidity for the period 1960 through the first six months of 1970, from one hospital serving this area, do not indicate any trends that are suggestive of adverse influences on human reproductive function that might be associated with herbicide use during the years 1965, 1966, 1968 and 1969.
>
> Even though the analytical data available to us apply only to the years 1969 and 1970, the rate of disappearance of these compounds in the environment leads us to believe that gross, protracted contamination was probably absent in prior years as well. Although samples of human tissues and body fluids, obtained through the cooperation of the medical profession in the area, are few in number, we believe the analytical results (which were all negative) are very probably representative.

VII. THE SWEDISH LAPLAND EPISODE

In the spring of 1970, Swedish newspapers reported an accumulation of sudden deaths of reindeer grazing in the Visttrask area of Lapland. Approximately 30 reindeer, mainly young animals, died within a week after a heavy, wet snowfall, without any previous signs of illness. It was also reported that about 10 reindeer cows aborted their fetuses. Examination of several reindeer by veterinarians showed inanition (empty stomachs). When given additional feed, the deaths stopped. The case was of particular interest since it was learned that the area where the reindeer grazed had been treated with a mixture of 2,4-D plus 2,4,5-T (2).

Analyses performed on liver and kidney samples (33,35) from one of the cows and three aborted fetuses noted above indicated traces of 2,4-D (0.2 to 0.5 ppm) and 2,4,5-T (0.3 to 1.0 ppm). Tree leaves contained 25 and 100 ppm of 2,4-D and 2,4,5-T respectively. However, no herbicides could be found in the ground vegetation. Although it was generally accepted that the deaths of the reindeer were attributed to starvation rather than exposure to 2,4,5-T and/or TCDD, Erne (34) initiated a controlled experiment on female reindeer and phenoxy herbicides.

Erne's experiment involved thirty pregnant reindeer, where half of the animals were given birch leaves from an area that had been aerially sprayed with one of the products that was used in the Visttrask area, the rest received untreated birch leaves. The average daily intake of leaves for both test and control groups was about 1 kg per animal, which for the test group corresponded to a daily dose of phenoxy acid of 1 mg/kg body weight. After the feeding experiment, the reindeer were sacrificed and necropsied just before the expected parturition.

During the course of the investigation, no clinical hematological or chemical signs were observed of injurious effects attributable to the sprayed leaves. The necropsy of the sacrificed animals showed nothing at all remarkable. All were pregnant (except one in the control group) and all the embryos were alive and normally developed. In histological investigations of the female reindeer and the fetuses, no pathological changes were observed that could be attributed to the prolonged consumption of sprayed leaves as fodder. Thus, Erne (34) concluded that the toxic manifestations noted in the Lapland incident were probably not caused by ingestion of herbicides.

Immediately following the report of reindeer deaths (and concurrent with press reports on alleged health effects from 2,4,5-T and TCDD in Vietnam), two cases of congenital malformations in human infants were also attributed to alleged exposure of pregnant women during application of phenoxy herbicides in Lapland forests (2). However, competent medical scientists at the Institute of Hygiene and the Teratological Laboratories of the Karolinska Institute of Stockholm and at the Institute of Human Genetics at Munster, Germany, were unable to find temporal or clinical evidence to suggest that the occurrence of these human birth defects was more than coincidentally related to the herbicide operations.

The publicity given to the Lapland incident resulted in additional reports of alleged adverse human health effects due to the phenoxy herbicides. For example, in early 1972, Swedish newspapers reported excess lung cancer mortality among railroad workers exposed to 2,4-D and 2,4,5-T. These reports prompted the Swedish National Board of Occupational Safety and Health to request an epidemiological evaluation of the stated excess mortality and its relation to herbicide exposure (10). The subsequent investigation as reported by Axelson and Sundell (10) in 1974 found that a slightly dose-dependent and significantly increased tumor incidence and mortality among workers exposed to the herbicide amitrol (3-amino-1,2,4-triazol) whereas those exposed to 2,4-D or 2,4,5-T had about normal tumor incidence and mortality. The study comprised 2,978 person-years at observation in the total cohort. The study has been recently reanalyzed with a case-control approach and through stratification on amitrol when considering the effect from phenoxy acids and vice versa (51). The results showed a possible and previously masked tumor inducing effect also from phenoxy acid.

By 1976 an intense debate was in progress in Sweden over the use of phenoxy herbicides. This debate prompted Hardell (45) to examine the occupational history of 87 patients who had malignant mesenchymal tumors and who had visited the oncological clinic in Umea during the years 1970-76. Nine of the 87 patients were forestry workers, four worked in farming and forestry and six in sawmills or the pulp industry. The implication by Hardell was that these 19 individuals were in occupations where exposure to phenoxy herbicides was relatively common. Based on the official statistics of Sweden, the expected fraction of tumors has been calculated for these occupations: the expentancy was 11 cases versus the 19 observed. Hardell (45) however, cautioned making any conclusion about the possible casual connection between exposure to phenoxy acids and contaminants and the occurrence of malignant tumors, solely on the basis of the reported cases.

In February 1977, the debate climaxed when the Royal Swedish Academy of Sciences organized a conference on "Chlorinated Phenoxy Acids and their Dioxins, Mode of Action, Health Risks and Environmental Effects" (31).

The conference participants concluded that (1) there was no evidence that dioxins could be formed in nature, (2) there was no evidence of bioaccumulation of TCDD at levels of application used in Sweden and (3) that if the concentration of TCDD can be kept below 0.1 ppm in all phenoxy formulations the risks involved can be disregarded and the safety factors based on the phenoxy acids themselves.

In the March 1978 WBBM television report on "Agent Orange: Vietnam's Deadly Fog", reference was made to a report from Sweden on birth defects (e.g., spina bifida) in children born to 65 women allegedly exposed to 2,4,5-T herbicide. The only reference to such an incident was that reported by Halling (44) in 1977. Halling studied the malformations in children born to mothers exposed to hexachlorophene soap during early pregnancy. All of the mothers were employed as nurses in a hospital and thus came in contact with the hexachlorophene in performance of this job. A group of 65 children born to this group showed six slight malformations and five severe malformations, whereas only one slight case in 68 children was observed in the unexposed group.

VIII. TE AWAMUTU, NEW ZEALAND EPISODE

The New Zealand episode had many similarities to the episode in Sweden; once the initial report was publicized, additional cases were forthcoming.

In January 1972, Sare and Forbes (72) reported the following in the New Zealand Medical Journal:

> "Sir, - Two babies, born within a month of each other at our local maternity hospital, had congenital defects incompatible with life. Both had a gross myelo-meningocele. Post-mortem was performed on only one and other congenital abnormalities were brought to light.
>
> What intrigued us was that the families concerned live on adjoining hilly country farms, where for several years aerial spraying has been carried out with a chemical called 2,4,5-T, designed to kill useless vegetation. Inquiries into the nature of this chemical revealed that it contains an impurity called dioxin, which is apparently one of the most powerful poisons ever discovered. It has been investigated in the United States, partially banned in all states, and totally in others. It was likewise banned in Vietnam when its potential danger was discovered....."

The suggested relationship between 2,4,5-T/TCDD and the two deformed babies quickly received national and international attention. Accusations that 2,4,5-T/TCDD were indeed responsible for the congenital defects soon appeared in articles in the United States (1, 32).

The circumstances surrounding these cases at Te Awamutu were thoroughly investigated by a subcommittee of the Agricultural Chemicals Board of New Zealand (6). In the subcommittee report it was noted:

> The women who gave birth to deformed babies had both been exposed to 2,4,5-T during pregnancy, one person by assisting at the airstrip during spraying and the second person by helping to free the spray truck which was stuck on the property and was exposed to 2,4,5-T when spraying was done to lighten the load. It was not possible to ascertain the degree of exposure in either case.

> The deformity common to both babies is spina bifida, caused by a failure of the end of the neural tube to close completely during early development. This deformity is one of the commonly occurring deformities, with overseas averages of about 1 per 1,000 total births. In New Zealand during the period 1964-70, 515 live births and 151 stillbirths affected with spina bifida were recorded. In the light of present embryological knowledge it may be stated that the neural tube is usually closed by the fourth week after conception and definitely by the sixth week. Medical records show that in one case, exposure to 2,4,5-T during the spraying operation occurred after the neural tube would have normally closed.
>
> It is concluded that in one of these cases the reported exposure to 2,4,5-T could not have caused the birth deformity. It is not possible to state definitely in the second case whether exposure to 2,4,5-T was in any way a factor causing the deformity, and thus the subcommittee was unable to arrive at any information of value to the general topic of 2,4,5-T toxicity to human foetuses.

In April 1977, the New Zealand televison program "Dateline Monday" suggested that the occurrence of "clusters" of neural tube defects in the South Taranaki, Northland and Waikato areas of New Zealand were related to the use of 2,4,5-T (61). The New Zealand Department of Health, Division of Public Health, appointed a committee of experts to investigate the allegations. In the Committee report, McQueen et al (61) noted that the three "clusters" represented 20 cases of birth defects. Seven of the cases were anencephaly (congenital defect of the cranial vault) and 13 were spina bifida (congenital defect of the bony encasement of the spinal cord). McQueen et al noted that although this group of defects may well have occurred entirely by chance, the possibility of a common causal factor must be considered.

After a thorough investigation of each of the 20 cases reported, McQueen et al (61) concluded:

> It is obvious from an inspection of the data for the three "clusters" that 2,4,5-T cannot reasonably be implicated in the causation of neural tube defects. It is true that in one or two cases there may have been some "exposure" to 2,4,5-T around the critical period. However, considering 2,4,5-T is the most used pesticide in New Zealand, this is not unexpected. In short, the data permit the conclusion that there is no evidence to implicate 2,4,5-T as a causal factor in human birth defects.

As a final note in relation to this episode, the following brief article appeared in the New Zealand Medical Journal (5):

> Publicity on certain chemicals as causation of malformations of the human fetus has been widespread. Some of the publicity has been sensation mongering and not all the remarks from the profession have been in keeping with a balanced assessment of scientific evidence. It is proper that there should be intelligent public awareness of the various environmental hazards that may come from the use of chemicals....in farming ....however, those who would write of their experiences in medical journals must remember that disasters are the staple of the sensation mongers in the news media industry.
>
> Until recent publicity there had been no suggestion that 2,4,5-T, which has been used for over 20 years in New Zealand, was responsible for congenital malfunctions either in man or in farm animals. It is the duty of the physicians (and scientists) who have any concern for science to attempt to make valid observations which can be repeated. In the problem at issue, fetal malformations are natures common mistakes which we have no desire to perpetrate or to increase, although they are the inevitable price that is paid for our place on the evolutionary scale. There are extensive gaps in our knowledge but they will be filled only by patient work. Unresolved problems of fetotoxicity can only be solved by accurate record keeping at all stages of pregnancy.

IX. DISCUSSION OF LITERATURE AND CONCLUSIONS

The episodes described in this chapter have provided much of our knowledge of the adverse effects to human health of the phenoxy herbicides, other chlorinated phenols and the associated dioxins. The only episodes however where TCDD was actually confirmed as a caustive agent were those involving some of the industrial accidents, the Eastern Missouri horse arena episode and the Seveso, Italy episode. Mercier (62) estimated that in the industrial accident in 1963 at the Philips-Duphar Company, Amsterdam, The Netherlands, up to 200g of TCDD were released into a factory hall. The incident in the horse arenas in Missouri may have involved 5,000g of TCDD (69). The quantity of TCDD involved in the Seveso, Italy episode has been estimated at 650-1,700g (69). In these three episodes, the TCDD was confined to a relatively limited area. The exposure of the people involved was from days (Philips-Duphar) to weeks (Seveso) to months (Missouri). Nevertheless, no human deaths were reported, although in both Missouri and Seveso, numerous animal deaths did occur. The clinical experience from these three episodes (and the other industrial episodes involving at least 1,000 individuals) support the opinion that patients without chloracne are extremely unlikely to have suffered the toxic effects of TCDD. In general, only in the most severe cases of chloracne has symptomatology persisted, admittedly for many years in a few instances.

The available scientific literature suggests that the episodes in Arizona, New Zealand and Sweden were primarily the result of emotionalism associated with zealous press coverage. Although each incident began subsequent to field applications of phenoxy herbicides, it was highly unlikely that the symptoms reported were attributable to actual pesticide or TCDD exposure. The behavior in the environment of 2,4,5-T and TCDD following normal field applications (see Chapter III) lends little credence to accusations that significant bioaccumulations occurred in humans to initiate the toxic symptoms reported. Furthermore, the absence of confirmed illness in domestic livestock or wildlife in these three episodes also addresses the issue of whether an actual toxic exposure occurred. Chapter IV defined the concentrations of herbicide and TCDD that were toxic to animals. The magnitude of the dosage required to elicite toxic symptoms in animals might be obtained only under the most extreme cases (e.g., spills or sequential repetitive applications). These extreme situations were not noted in the episodes in Arizona or New Zealand.

The human responses associated with these episodes show a similarity to what occurred in Michigan involving exposure to polybrominated biphenyls (PBB). In 1973 and 1974, more than 10,000 Michigan farm residents were exposed to PBB when several hundred pounds were accidentally introduced into a nutritional supplement that was subsequently fed to numerous herds of dairy cattle. Budd et al (18) conducted an epidemiological study in an effort to determine whether or not exposure to PBB had caused illness in Michigan residents. Three groups were invited to participate in a prospective cohort study: (1) all persons who had been identified as living on PBB-contaminated farms at the time of quarantine; (2) all persons who had received food products directly from such farms; and (3) workers and their families who had been exposed occupationally to PBB in a chemical manufacturing plant. All subjects were administered a questionnaire requesting information on the occurrence in the years before and since 1973 of 17 symptoms and conditions potentially related to PBB. Venous blood samples were also obtained on the subjects. An evaluation of dose-response relationships revealed that symptom-prevalence rates were higher in persons with no detectable PBB in serum than in those with measurable quantities. These observations suggested that factors other than PBB absorption were responsible for the production of symptoms and that selection factors (e.g., selecting from a list of given symptoms by the subject) may have played an important role in the observed distribution of complaints.

The episodes in Arizona, New Zealand and Sweden all occurred in the same time period; a period when numerous articles appeared in the world press on the alleged human health effects of Herbicide Orange and TCDD in South Vietnam. The effects these articles had on the actual episode can only be speculated.

The wide publicity that was given to the use of defoliants, especially Herbicide Orange in South Vietnam, appeared to have exceeded concerns of human health or the environment. Political issues may certainly have been a major reason for much of this publicity. Consider, for example, the data in Table 1 of this chapter; more 2,4,5-T and hence TCDD, was disseminated in the United States during the same period than in South Vietnam. If the assessment of canopy penetration is reasonably accurate in Chapters I and III, then the actual ground-level deposition of Herbicide Orange in South Vietnam (1.4 pounds 2,4-D/2,4,5-T per acre) would have been approximately equal to the concentrations of herbicides encountered at ground-level following brush applications in the United States.

The Committee on the Effects of Herbicides in South Vietnam of the the National Academy of Sciences (21) attempted to assess the effects of propagandistic activities on the attitudes of the South Vietnamese towards the use of herbicides. The following statements are quotations from the 1974 report:

> Our findings indicate that there is a major dichotomy between the views of the rural population and those of the urban middle-sector regarding the use of herbicides in SVN. Contrary to what might be expected, the herbicide missions are much less emotional issue among the peasants, who bore the brunt of the effects, than it is among urban intellecturals for whom it has become a symbol.
>
> Despite extensive propaganda and counter-propaganda campaigns waged by the RVN and the NLF, peasant views regarding herbicide effects seem to be based upon their own experience. The RVN stressed that herbicides were used as a military measure to deprive the guerrillas of their hiding places, that the herbicides might damage crops but could also have beneficial effects, and that people and livestock would not be adversely affected by spraying. NLF statements emphasized the dangerous nature of herbicides. They claimed that the chemicals caused the death of people as well as livestock and crops, resulted in increased numbers of miscarriages and stillbirths, and caused numerous diseases, especially leprosy and conjunctivitis. Further, it was said that the U.S. had deliberately introducted "chemical bacteria" into the spray which could penetrate peoples bodies and cause disease. The fact that the villagers did not appear to subscribe blindly to the propaganda claims of either side does not mean that they lacked political opinions nor that they were uninfluenced by information derived through the mass media. Rather it seems to mean that their opinions on this issue came mainly from their own observations.

The degree to which the above referenced propaganda influenced world opinion is illustrated by Dmitriyev (28) in articles published in 1974 in a Russian medical journal. The following quotation is a translation from that journal:

> Often in South Vietnam, chemical substances were used not only in the forest regions but also close to populated areas; this resulted in injury to a considerable part of the peaceful population. According to the data of the Provisional Revolutionary Government of the Republic of South Vietnam in 1961-1969, 1,293,000 persons were subjected to the effect of poisonous chemicals. In the first ten months of 1970, 185,000 cases of persons being poisoned were recorded. Three hundred persons died and a significant number of those injured became chronic patients.
>
> Persons injured by herbicides and defoliants noted perceiving a sharp odor of chlorine or DDT, sharp pain, burning in the nasopharynx and sneezing (91%), crying and vomiting (73%), headache and vertigo (38%), a burning sensation in the area of the eyelids and the skin (41%). These clinical symptoms were apparent after a 24-hour incubation period. Improvement in the patients, if they did not die, began after 3-4 days. However, they continued to suffer from asthenic symptoms in the form of sleeplessness, sexual weakness, and weakening of the vision.

Similar quotations are available in American or European literature. Mercier (62), in reviewing the literature on TCDD for a conference in Milan, Italy in 1976, stated of the National Academy of Science Report (21):

> Considerable information is contained in a NAS report (1974) about the effects of herbicides, and especially 2,4,5-T, so-called "Agent Orange" and the contaminant TCDD on humans, animals and vegetation in Vietnam where they have been used during military herbicide operations. It contained reports of death to children, diarrhea, skin rashes looking like insect bites, and abdominal pain following spray missions. The use of the materials also significantly increased the incidence of congenital malformations among children.

The point to be made is that the scientific studies that have been conducted in Vietnam; Globe, Arizona; Eastern Missouri, Sweden; New Zealand; Seveso, Italy; and the numerous industrial accidents do not document deaths of children or adults due to the herbicides or TCDD, nor do they substantiate increased incidence of congenital malformations among children. The reports published by North Vietnamese scientists provide insufficient data on which to draw contrary conclusions.

X. SUMMARY

Increased industrial production of the phenoxy herbicides paralleled the rapid acceptance of these materials in world agriculture. The demands upon the industrial production however, resulted in at least 23 industrial incidents involving over 1,100 people (almost all adult males). Although medical examinations were initially conducted on these individuals, few long-term studies are available.

The use of herbicides by the United States military in South Vietnam precipitated numerous allegations of adverse health effects upon the human population. Review of the scientific literature of the few available studies conducted in Vietnam do not confirm the allegations.

Episodes of TCDD poisoning in Eastern Missouri in 1974 and in Seveso, Italy in 1976 resulted in adverse effects to primarily women and children. Although the acute symptoms of poisoning have dissipated, long-term effects remain to be determined.

Episodes of alleged poisoning from 2,4,5-T and TCDD in Globe, Arizona (1969-70), Sweden (1970) and New Zealand (1972) occurred in a period of time when intense publicity was given to the use of herbicides in South Vietnam. The available scientific studies of these incidents suggest that factors other than herbicide exposure may have been responsible for the symptoms reported.

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CHAPTER VI

HUMAN EFFECTS OF HERBICIDE ORANGE

I. INTRODUCTION

This chapter will discuss the human effects of Herbicide Orange. There has been considerable medical literature published on the constituents of Herbicide Orange, i.e., 2,4-D, 2,4,5-T, and the contaminant TCDD. The pharmacokinetics of these chemicals will be discussed along with their adverse effects. Most of the reports in the literature have involved occupational experiences. However, several episodes involving general populations from selected localities throughout the world will also be discussed.

II. PHARMACODYNAMICS

Little work has been done regarding the pharmacodynamics of 2,4-D, 2,4,5-T or TCDD in humans. The available studies are summarized below.

A. Percutaneous Entry of Phenoxy Herbicides

Feldmann and Maibach (27) in 1974 in studies using radioactive tracers showed that 2,4-D was able to penetrate the skin. Indirect evidence resulting from the numerous occupational exposures to 2,4-D and 2,4,5-T (and TCDD) in industry and herbicide spraying described later further supports percutaneous entry.

B. Ingestion of Phenoxy Herbicides

Kohli et al (46, 47) in two separate studies gave purified 2,4-D and 2,4,5-T in capsules to human volunteers. Each herbicide was orally administered to six men as the acid at a dose level of 5 mg herbicide per kg body weight (mg/kg). The 2,4-D was quickly absorbed and appeared in the plasma within one hour after ingestion. Seventy-five percent of the administered dose was excreted unchanged in the urine within 96 hours (h). The 2,4,5-T was also readily absorbed, being present in the plasma one hour after ingestion. After 96 h, 63 percent to 72 percent of the herbicides had been excreted unchanged by the kidney. Plasma levels peaked between seven and twenty-four hours for both 2,4-D and 2,4,5-T and the half-lives for plasma clearance were 33 and 18 h respectively. In a study by Saueroff et al (70) in 1977, five male humans ingested 5 mg/kg of 2,4-D. Essentially all was absorbed from the gastrointestinal tract. It was eliminated from the plasma with an average half-life of 11.6 hours and from the urine with an average half-life of 17.7 hours. Eighty-two and three tenth percent was excreted unchanged and 12.8 percent in a conjugated form for a 95.1 percent total recovery. Utilizing this rate of clearance, 99 percent of the steady state would be reached in about three days making body accumulation of repeated exposure unlikely. Gehring et al (28) in 1973 and Matsamara (52) in 1970 found similar results in comparable studies of 2,4,5-T. It should be noted that no short-term adverse effects were found in any of the above studies with 5 mg/kg being the highest dose.

The fate of Silvex [2-(2,4,5-trichlorophenoxy) propionic acid] was studied by Saueroff et al (71). Seven men and one woman ingested a dose of 1 mg/kg. Peak plasma levels were reached two to four hours after ingestion. The Silvex was excreted in the urine both in the unchanged and conjugated forms. The mean recovery in the urine was 64 percent of the orally administered dose after 24 hours and 79.8 percent after 144 hours. Recovery of Silvex in the feces accounted for not more than 3.2 percent of the administered dose. The half-life for plasma clearance was biphasic, 4.0 ± 1.9 h and 16.5 ± 7.3 h for initial and terminal periods respectively. No adverse effects were noted.

Park et al (60) described clinical and pharmacokinetic observations in a 39-year-old male following ingestion of the amine salts of 2,4-D and [2-(2-methyl-4-chlorophenoxy) propionic acid] (MCPP).

Following forced alkaline diuresis, the plasma half-half of 2,4-D was greatly reduced from 220 to 4.7 h. The renal clearance of 2,4-D increased up to greater than 100-fold during the period of alkaline diuresis. Urinary recovery studies confirmed absorption of about 70 ml of the herbicide. Although the patient initially demonstrated a mild proximal neuropathy and myopathy, full recovery occurred in two months.

C. Tissue Analyses for the Phenoxy Herbicides

Levels of phenoxy herbicides found in human tissue or body fluids following ingestion of a fatal dose are shown in Table 1. The data are reported in parts per million: it was assumed that all tissues (removed during the autopsy) were analyzed on a fresh weight basis and that 1 ml of blood or urine was equal to 1 g. Frequently, no description of the handling procedures was reported; thus, fluid may have been present within the organ (e.g., liver) at the time of the analyses. This fluid contamination may have resulted in high values for a given tissue.

Coutselinis et al (20) in 1977, reported on the analyses of herbicide in the organs of a woman who died 16 hours after ingesting a large dose of a mixture of 2,4-D and 2,4,5-T. Levels of the two herbicides found in selected tissues at the time of death are shown in Table 1. The formulation ingested was a mixture in a 3:2 ratio, 2,4-D to 2,4,5-T.

Nielson et al (56) reported a more complete investigation of herbicide residue in body tissue resulting from an autopsy of a 23-year-old male who ingested 2,4-D. The levels of 2,4-D in parts per million for selected tissue are also shown in Table 1.

The herbicide 2-methyl-4-chlorophenoxyacetic acid (MCPA) has been associated with two deaths. Johnson and Koumides (39) reported the death of a 65-year-old man following the ingestion of 250 mg MCPA/kg body weight.

TABLE 1

Levels (part per million) of Phenoxy Herbicides in Human Tissue or Body Fluid Following Ingestion of Fatal Dose[a]

Source	Patient	Herbicide	Dose (mg/kg)	Time Ingestion - Death	Level of Herbicide (Parts Per Million) in Tissue[b]									
					Blood	Urine	Liver	Kidney	Brain	Spleen	Muscle	Heart	Gastric Washings	Fatty Tissue
Coutselinis et al. (20)	Young Woman	2,4-D/ 2,4,5-T	"Large"	18 hrs	826 182		210 48	82 22		12 5				
Nielson et al. (56)	23 Yr Old Man	2,4-D	>80	24 hrs	669	264	183	63	13	134	70			83
Dudley and Thapar (23)	76 Yr Old Man	2,4-D	>2,000[c]	5 days	58		408	194	93		118			
Johnson and Koumides (39)	65 Yr Old Man	MCPA	250	20 hrs	180	800							2,500	
Popham and Davies (63)	32 Yr Old Man	MCPA	440	20 hrs	230	970	146		33			154	3,000	

[a] Tissue/fluid removed during autopsy.

[b] To convert parts per million to mg/dl or to mg/100 mg, multiply tabulated values by 0.1

[c] The 55 kg patient consumed one pint of a presumed ester formulation (kerosene-like, water insoluble formulation) of 2,4-D. Ester formulations containing the least amount of active ingredient 2,4-D are two pound/gallon formulations. If a pint contained 113 g 2,4-D acid, then the dose would have been >2,000 mg 2,4-D/kg body weight.

Popham and Davis (63) report the death of a 32-year-old man following a MCPA dose of 440 mg/kg. The levels of MCPA found in selected organs or body fluids following death are reported in Table 1. The high level of MCPA in the urine suggests that this herbicide, like 2,4-D, was rapidly excreted unchanged by the kidney.

D. Pharmacodynamics of TCDD

There is almost a complete lack of information concerning the pharmacodynamics of the dioxins in man. In November 1977, Fanelli (2) in a letter to the Mario Negri Institute of Pharmacologic Research in Italy found no TCDD in samples of the liver, mesenteric fat, or cerebral fluid in the necropsy of a woman who had been included in a follow-on study of the Seveso, Italy, TCDD episode. The lower limits of detection were 0.4 ng TCDD/ml of fluid and 0.25 ng/gm of tissue. The cause of death was not given.

Reggiani (65) described the case of a 55-year-old woman who died of pancreatic carcinoma with liver involvement seven months after the Seveso episode. Children living with her suffered severe caustic burns of the skin and, subsequently, chloracne. Neither the patient nor the mother of the children developed chloracne. It is almost certain that the entire family ate food contaminated with TCDD. TCDD detected in the analysis of tissue obtained during the autopsy is shown in Table 2. No TCDD was found in the same tissues taken from autopsies of three persons who were certainly not related to a TCDD exposure. The samples were run concurrently with those of the case described above.

III. ADVERSE EFFECTS

A. Limitations of Referenced Studies

There is considerable information in the world literature regarding the adverse effects of 2,4-D, 2,4,5-T, 2,4,5-trichlorophenol (TCP) and TCDD in humans. Most of it is the result of studies on worker experience, industrial accidents or individuals poisoning. Unfortunately, there are very few controlled studies and only generalizations can be made regarding a cause-effect relationship. In most cases all that can be said is that an association exists. There are several other important limitations of the studies that must be kept in mind when reviewing them. These include:

1. The populations were biased toward the adult male of working age.

2. Examinations were post-exposure, and therefore, pre-existing disease often was not known or reported.

3. Exposures frequently were to mixtures and, therefore, one cannot be certain which chemical produced which effect.

4. An accidental or intentional ingestion or an exposure from an industrial accident would result in a dose much higher than would be expected in the general population in the region of a herbicide spraying program.

TABLE 2

TCDD Levels in a Human Body

Date	Sample	Origin	Quantity	Limit of Detection	Recovery	TCDD[a]
[b]28, 7, 7	Liver	Autopsy	10 g	10 PPT	64%	0.15 PPB
	Fat	Autopsy	10 g	10 PPT	59%	1.84 PPB
	Pancreas	Autopsy	5 g	10 PPT	59%	1.04 PPB
	Lung	Autopsy	10 g	10 PPT	60%	0.06 PPB
	Kidney	Autopsy	10 g	10 PPT	60%	0.04 PPB
	Brain	Autopsy	10 g	10 PPT	60	0.06 PPB

[a] - Total body weight: kg 70 - Calculated total amount at time of death: 40 µg

[b] - Vacuum Generator Micromass Laboratory, Altrincham (Manchester, U.K.)

Source: Reggiani (65).

5. Although the routine occupational exposure would in most cases be at a dose rate lower than that of accidents, the exposure would be prolonged, effectively raising the total dose.

6. The actual dose received in most instances was not known.

B. Phenoxy Herbicides That Do Not Contain TCDD

As was explained in a previous chapter, TCDD is a contaminant of phenoxy herbicides made from TCP. TCP is not a precursor of 2,4-D. This permits the evaluation of health effects of 2,4-D (or 2,4-D-like herbicides) as an entity separate from TCDD.

1. Experimental Exposure to 2,4-D

There have been at least three reports of no-effect exposure where the precise dose was known. Assouly (4) in 1951 reported on a man who ingested 0.5 g of 2,4-D daily for three weeks without adverse effects. Kohli (46) in 1974 in his pharmacodynamic study of 2,4-D reported no effect after a single oral dose of 5 mg/kg. In 1962, Seabury (74) treated two cases of disseminated coccidiomycosis with 2,4-D. The first patient received a total of 40 mg of the sodium salt by intramuscular injection over a period of four days. The patient died on the fifth day without evidence of 2,4-D toxicity. In the second patient, approximately 13 g were given intravenously over a period of one month, the last 2 g in one dose. No adverse effects were noted. When the dose was increased to 3.6 g over a period of two hours the patient became semi-stuperous and exhibited fibrillary movements about the mouth and in both hands and forearms. The stupor deepened to a point where the patient responded only to painful stimuli. Forty-eight hours after the dose was given he returned to his pre-reaction state. There was no evidence of neurologic or muscular change in the next seventeen days after which he died from the primary disease.

2. Exposure in the Production of 2,4-D or MCPA

Bashirov (8) in 1969, examined 292 workers including 44 women employed in the production of the amine salt and the butyl ester of 2,4-D. This report is of particular significance in that the butyl ester is the form found in Herbicide Orange. Table 3 shows the various responses along with the percentage of occurrence. Several organ systems were involved with emphasis on headaches, the asthenic syndrome, and gastrointestinal complaints. Fifty persons from the above group were selected for controlled studies involving the liver and stomach. Bashirov indicated that there were significant differences between the control and test groups in amount of gastric secretion and the antitoxin and carbohydrate functions of the liver. In addition, they noted a correlation between the length of service and the changes in the functional state of the stomach. The authors did not state their level of confidence.

Telegina and Bikbulatova (78) in 1970 reported on 158 workers employed in the production of MCPA. Telegina and Bikbulatova found contact

TABLE 3. Distribution of symptoms in 292 workers employed in the production of the amine salt and the butyl ester of 2,4-D.

	Symptoms	Percent of workers describing symptoms
1.	Weakness, fatigability, headaches	63
2.	Asthenic Syndrome with vegetative dysfunction	61
3.	Anorexia, bitter taste in mouth, dyspepsia abdominal pains, constipation	51.7
4.	Vertigo	33
5.	Dyspnea on exertion	26.7
6.	Tachycardia, precordial pain	17.8

Source: Bashirov (8)

dermatitis or history of same in 55 individuals in the first examination and in 65 in a second examination a year later. Irritation of mucous membranes was also found in a majority of these individuals.

3. Accidental or Intentional Exposure to 2,4-D, MCPA, 2,4-DP or MCPP

Another major group of persons exposed to 2,4-D or the analogs MCPA, 2,4-DP [2,4-dichlorophenoxy) propionic acid] and MCPP, are those involved with accidental or intentional ingestion of the substance. Table 4 is a summary of many such cases along with the estimated dose of herbicide (where available), major effects and outcome of the intoxication.

Popham and Davies (63) reported the case of a 32-year-old man who ingested an estimated dose of 440 mg MCPA/kg. There were signs of severe meningoencephalitis including grand mal and focal seizures with death within hours. Necropsy showed no evidence of damage to the gastrointestinal tract, but the liver showed signs of early necrosis. The brain and meninges showed marked congestion but otherwise were normal. Johnson and Koumides (39) described a similar MCPA episode without the severe central nervous system signs and with death in hours. The dose was estimated at 250 mg/kg.

Nielson et al (56) published a paper describing a 23-year-old man who committed suicide by ingesting an unknown amount of 2,4-D. Tissue analysis indicated, however, that at least 80 mg/kg must have been absorbed. Unlike the cases of Johnson and Koumides (39) and Popham and Davies (63), this subject was in good physical health prior to the ingestion. The others were suffering from chronic illnesses. There was evidence that this subject had at least one convulsive episode before dying, implicating the central nervous system. In the necropsy, small amounts of 2,4-D were found in the brain tissue (see Table 1). There was also evidence of degeneration of ganglionic cells in the brain. If the degeneration was due to 2,4-D and not hypoxia, it would have indicated that the cellular elements of the central nervous system were quite sensitive to 2,4-D as the tissue analysis showed the brain to have a much lower level of herbicide when compared with other organs of the body.

Other episodes of poisoning by 2,4-D or MCPA have been reported by Jones et al (40), Berwick (12), Brandt (15), Dudley and Thapar (23), and Park et al (60). Findings, other than those involving the central nervous system, included abnormal enzyme levels, anemia, thrombocytopenia, skeletal myositis with myoglobinuria, myocardial irritability, loss of color vision, peripheral nervous system disorders, pulmonary edema, and renal disorders. The subject reported by Dudley and Thapar (23) died; the remainder survived with varying degrees of recovery. The case reported by Brandt (15) had a complete recovery after an estimated dose of 300 to 600 mg/kg; however, this individual had ingested a mixture of 2,4-D and 2,4-DP.

The case reported by Berwick (12) was noteworthy because the individual involved accidentally ingested a dose of 110 mg 2,4-D/kg. The herbicide was formulated as the isooctyl ester of 2,4-D. Although the individual demonstrated numerous symptoms (Table 4), he fully recovered.

TABLE 4

Distribution of Adverse Effects in Case Reports Following the Ingestion of Non-TCDD Containing Phenoxy Herbicides

Source	Date Reported	Exposure	Herbicide	CNS Irritation	CNS Depression	Peripheral Neuropathy	Hematopoetic Depression	Myopathy	Gastrointestinal Irritation	Nephropathy	Hepatotoxicity	Special Senses Alteration	Cardiopathy	Outcome
Popham and Davies (63)	1964	440mg/kg	MCPA	+	+									Death
Johnson and Koumides (39)	1965	250mg/kg	MCPA		+									Death
Nielson et al. (56)	1965	>80mg/kg	2,4-D	+	+				+					Death
Jones et al. (40)	1967	<1900mg/kg	MCPA	+	+		+		+	+	+			Full Recovery
Berwick (12)	1970	110mg/kg	2,4-D	+	+			+	+	+	+			Full Recovery
Brandt (15)	1971	300-600 mg/kg	2,4-D/ 2,4-DP		+	+	+		+		+	+		Residual Peripheral Sensory Defect
Dudley and Thapar (24)	1972	>2000mg/kg	2,4-D		+			+	+	+	+		+	Death
Park et al. (60)	1977	Unkn	2,4-D/ MCPP		+	+		+	+					Full Recovery
Total Number of Reports Listing Effect				4	8	2	2	3	6	3	4	1	1	

The patient was routinely observed over a three year period and no signs of peripheral neuropathy occurred.

4. Exposure to 2,4-D in Spray Operations

A fourth group of exposed individuals is those who were involved in spraying operations contacting either the spray or the liquid. Table 5 summarizes these cases where individuals were exposed to 2,4-D.

In 1959, Goldstein et al (31) first reported on three patients who developed peripheral neuropathies manifested by pain, paresthesias and paresis. There had been previous skin contact with liquid 2,4-D indicating a probable percutaneous route of entry. Recovery from the neuropathy was incomplete for the three patients during the periods of observation which were 1, 2 and 3 years, respectively for a 65-year-old male, 50-year-old female and a 52-year-old male.

The 65-year-old male was exposed during the course of spraying a field with an ester of 2,4-D wetting his arms and legs. He was reported to have been in ill health prior to exposure. The 50-year-old female was exposed twice, one year apart, to an ester of 2,4-D wetting hands and legs. The 52-year-old male was exposed first when he spilled 60 ml 2,4-D ester on his arms and failed to wash it off. His second exposure was two months later, wetting his legs with the same formulation.

In 1961, Monarca and di Vito (55) reported a case where the entry route may have been at least partially respiratory, the subject having stayed downwind during much of the spraying operation. The immediate toxic symptoms consisted of asthenia, autonomic hyperactivity, gastrointestinal irritation and alterations of the central nervous system. Some days later he developed a hemorrhagic enterocolitis. After a period of five months recovery was complete except for hyporeflexia of the lower limbs.

Berkley and Magee (11) described the development of peripheral neuropathy in a 39-year-old farmer who had significant hand contact with 2,4-D. At the end of one year the only residual effect was mild hypoalgesia on the fourth and fifth fingers of the right hand. Todd (80) reported a case in which the subject presented with anemia and leukopenia as well as peripheral neuropathy. The subject had two separate contacts with liquid 2,4-D experiencing gastrointestinal symptoms each time. The neuropathy lasted almost two years.

In 1966, Tsapko (81) reported headache, retrosternal pain, general weakness, vertigo, nausea, vomiting, and mild leukopenia in a group of field workers who entered an area immediately after it was sprayed with 2,4-D.

Kotlarek-Haus et al (48) described an autoimmune hemolytic anemia in a pesticide applicator. However, DDT, Lindane and Fenthion were routinely sprayed by this individual, as well as was 2,4-D

TABLE 5

Distribution of Reported Adverse Effects Following Exposure of Field Workers and Applicators to 2,4-D

Source	Year of Episode	Number Of Cases	2,4-D Formulation	Primary[a] Route of Exposure	CNS Irritation	CNS Depression or Dysfunction	Peripheral Neuropathy	Hematopoetic Depression	Myopathy	Gastrointestinal Irritation	Nephropathy	Cardiopathy	Asthenia	Dermatitis	Period of Observation	Remarks
Goldstein et al. (31)	1955	3	Ester	Percut	+		+			+	+			+	3 Yrs	One case of neuropathy for 3 Yrs
Monarca and di Vito (55)	1960	1	Sodium Salt	Inhal		+				+	+		+		5 Mos	Residual hyporeflexa
Todd (80)	1960	1	N/A[b]	Percut		+	+	+		+					2 Yrs	Full recovery but neuropathy lasted two Yrs
Berkley and Magee (11)	1961	1	Amine Salt	Percut			+								1 Yr	Mild hyperalgesia in two fingers
Tsapko (81)	1966	Group	Sodium Salt	Percut		+		+		+			+		N/A	No comment
Wallis et al. (87)	1966	1	N/A	Inhal			+		+						2 Yrs	Full recovery
Paggiaro et al. (58)	1972	1	Ester	Inhal		+			+		+	+	+		1 Mo	Full recovery
Total Number of Reports Listing Effect					1	4	4	2	2	4	3	1	3	1		

[a] Percut = Percutaneous; Inhal = Inhalation

[b] Formulation description not available.

Sare (69) reported on a subject who complained of diplopia toward the end of days in which he sprayed 2,4-D.

In 1974, Barthel (7) related three cases of pulmonary fibrosis in workers engaged in weed control programs using MCPA. It is more probable, however, that the fibrosis was related to the carrier substances which were slatemetal, kaolin, and talcum.

In a letter to the editor, Taylor (76), reported a suicide in a young farmer who became depressed over an illness possibly resulting from exposure to 2,4-D and 2,4,5-T. The illness was not specified nor was it clear whether the depression was a primary response to the herbicides or entirely secondary to the illness. However, this was the only reference found in which a psychiatric disorder was attributed to 2,4-D.

Paggiaro et al (58) described an individual intoxicated by inhalation of 2,4-D. The individual manifested headaches, constipation, urinary incontinence, myalgia, muscular hypotonia, proteinuria and tachyarrhythmia. Despite these numerous symptoms, the patient fully recovered in one month.

Palva et al (59), in 1974, reported a case of aplastic anemia in a 64-year-old farmer after exposure to MCPA. Recovery was complete after five months.

C. Trichlorophenol (TCP), 2,4,5-T and TCDD

Since TCDD is formed in the production of TCP (see Chapter V), both TCP and 2,4,5-T are contaminated with TCDD. As a result, TCDD must be considered when discussing either TCP or 2,4,5-T. Although other dioxins are usually formed in the production of pentachlorophenol (PCP), small amounts of TCDD may also be produced and, therefore, exposure to PCP will be included in this section.

1. Industrial Exposure and Symptomatology

Since the first commercial production of 2,4,5-T there have been numerous industrial episodes involving exposure to TCP, 2,4,5-T and TCDD. Chapter V discussed these industrial episodes in depth. Fifteen of the 23 episodes recorded in the literature were apparently occupational exposures that occurred during industrial production of chlorinated phenols. However, on eight occasions, explosions occurred and personnel were exposed during the clean-up of the accident or from subsequent exposure to an improperly decontaminated workshop.

The symptomatology reported for various occupational episodes are presented in Tables 6, 7 and 8. Table 6 is a summary of the organ systems affected during episodes of occupational exposure to chlorinated phenols and/or TCDD. Table 7 is a summary of the signs, symptoms and disorders reported for these episodes. Table 8 is a summary of special clinical studies conducted in support of physical examinations given to selected

TABLE 6

Organ Systems Reported Affected After Occupational Exposure to PCP, TCP, 2,4,5-T or TCDD

Source	Chemical	Number Examined	Skin	Liver	Urinary Tract	Lower Respiratory	Mucuous Membranes	Central Nervous System	Peripheral Nervous System	Autonomic Nervous System	Heart	Pancreas	Special Senses	Joints	Vascular	Comment
Baader and Bauer (6)	PCP	17	17[a]	6	2	7					4			4		Examined June 1950, 16 months after last exposure
Bauer et al. (9)	TCP/2,4,5-T	9	8	4	1	3	5	6	9	5	2					31 workers exposed. Examined 5 years after exposure ceased.
Bleiberg et al. (14)	TCP/2,4,5-T/2,4-D	29	21	+[b]												
Poland et al. (62)	TCP/2,4,5-T/2,4-D	73	48	6		20	23				3		10			Same plant as Bleiberg 1964 after improved conditions.
Dugois et al. (24)	TCP	17	17				+									Chloroform odor from skin.
Hardell (33)	Phenoxy Acid	87														
Kimmig and Schulz (44)	TCP/2,4,5-T	31	31	3			+			+						Same plant as Bauer 1961.
Kramer (49)	2,4,5-T	64	3	1	1	10	8		1	4						No significant difference in findings from control.
Jirasek et al. (37)	PCP/2,4,5-T	78	76	+				+	+	+		+				2 persons had porphyria without acne.
Jirasek et al. (38)	PCP/2,4,5-T															Subjects taken from group examined in Jiracek et al. (37)
Pazderova et al (61)	PCP/2,4,5-T	55	53	+	+	+		4	13	+					+	
Miura et al. (54)	PCP/2,4,5-T	25	+													
Oliver (57)	TCDD	3	3					1	1	1				1		Lab workers synthesizing TCDD.
Ter Beek et al. (79)	2,4,5-T		+							+						
Zelikov and Danilor (88)	TCP	1	1													
Number of cases in which organ system affected[c]		489	278	20	4	40	36	11	24	10	9	0	10	5	+	

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[a] Number entries in table reflect the number of cases in which a disorder of the organ system was reported.

[b] + = Organ system involvement reported; however, number of cases not given.

[c] Numbers do not include cases represented by "+" and totals may represent some double counting due to overlap of studies by Jirasek et al. and Pazerova et al.

TABLE 7
Signs, Symptoms, and Disorders Reported After Occupational Exposure to TCP, 2,4,5-T or TCDD

Source	Headaches	Sensory Nerves and Tracts	Neuralgia or Myalgia	Paresis	Hemorrhage	Porphyria	Hyperpigmentation or Hirsutism	Acne	Fetal Disorders	Cancer	Asthenia	Other Psychiatric	Abdominal Pain or Pressure	Anorexia, Nausea Vomiting, Diarrhea	Death
Baader and Bauer (6)			8	2				17			4				
Bauer et al. (9)	4[a]	3	6	9			5	8			9	6	5		
Bleiberg et al. (14)						11	18	20							
Poland et al. (62)	8	2		7		1	30	48					+	22	
Dugois et al. (24)	+[b]							17			+		+	+	
Hardell (33)										87					
Kimmig and Schulz (44)				+				31			+				
Kramer (49)	3										4	2			
Jirasek et al. (37)	+		+	+		12	19	78		2	+	+		+	3
Jirasek et al. (38)		+				+						+			
Pazderova et al. (61)			+	+		23	+	53		2	27	8	+	+	3
Miura et al. (54)								+							
Oliver (57)	2	1	1				3	2			3	1	1	1	
Ter Beek et al. (79)	+					+		+			+	+		+	
Zelikov and Danilov (88)								1							
Total number of cases reported[c]	17	6	15	18	0	47	75	275	0	91	47	17	6	23	6

[a] *Number entries in table reflect the number of cases in which sign, symptom or disorder was* reported. [b] + = Sign, symptom or disorder reported but number of cases not given.

[c] Numbers do not include cases represented by "+" and totals may represent some double counting due to the overlap to studies by Jirasek et al. and Pazderova et al.

TABLE 8

Special Clinical Studies Following Occupational Exposure to TCP, 2,4,5-T or TCDD

Source	Liver Funct	Renal Funct	Carbohydrates	Lipids	Proteins	Blood Elements	EEG	Blood Pressure
Baader and Bauer (6)	6[a]	2				6		
Bauer and Schulz (9)	2	1					6	0
Poland et al. (62)	2	1	6	7		2		4
Kramer (49)	7	1	1		1	5		14
Jaracek et al. (37)				+[b]				
Pazderova et al. (61)[c]	11		11	37	8		9	
Oliver (57)				3				
Total number of cases with abnormal study[d]	28	5	18	47	9	13	15	18

[a] Number entries in table reflect the number of cases in which the special study was reported as abnormal.

[b] + = Special study reported as abnormal but number of cases not given.

[c] The results include studies reported in Jiracek et al. (40). The two studies complement each other.

[d] = Numbers do not include cases represented by "+".

individuals following or during occupational episodes. The data in these tables are probably representative of the over 520 individuals that were reported in Chapter V to have been medically examined following the various occupational episodes.

Approximately 600 individuals were adversely affected by exposure to TCDD following eight reported industrial accidents (see Chapter V). These individuals were either involved in the accident, responsible for clean-up after the accident, or returned to work in the plant following the accident. Table 9 is a summary of organ systems affected after an exposure to TCP and TCDD following these industrial accidents. Table 10 is a summary of the signs, symptoms and disorders noted in the individuals following exposure to TCP and TCDD. Table 11 is a summary of the few available data on special clinical studies on those individuals involved in the industrial accidents.

Armstrong et al (3) and Robson et al (67) have reported on extensive medical data (organ systems affected, symptoms, disorders and clinical examinations) from newborn infants exposed to sodium pentachlorophenate in a hospital episode of PCP poisoning. Since these data involved newborn infants and PCP in a hospital environment, they were not included in the Tables.

The data in Tables 6 thru 11 list the effects reported in the industrial environment where TCDD may be produced in the course of trichlorophenol production. The absolute numbers must be looked upon with caution for the reasons expressed earlier. There were no controls and preexisting conditions in most cases were not described in the articles. This limitation is demonstrated nicely by the study of Reggiani in 1977 (64) on the workers of the ICMESA plant in Seveso, Italy. As will be explained in more detail later there appears to be minimal if any development of systemic disorders if chloracne or a history of the same is not also present (64, 65). (Personal communication: Crow, K. D., Princess Margaret Hospital, Swindon, England. Holder, B. B., Dow Chemical Company, Midland, Michigan.) Out of 176 ICMESA workers examined immediately after the accident and more thoroughly four weeks after, only one displayed a doubtful case of chloracne. Yet, there were 29 subjects with liver disorders, 28 with lower respiratory problems, and nine with disorders involving the heart. In this case, the caustic products and TCDD exited the plant through a stack resulting in minimal, if any, exposure to the workers in the plant. This is contrasted with other accidents where the formed material remained in the plant providing major exposure to TCDD. If the premise that chloracne will be present before or during the time systemic symptoms are present is accepted, the abnormalities seen in this case must be due to some etiology other than TCDD. It is a matter of speculation as to why the one worker developed chloracne. There are at least two possibilities. He may have had a low threshold or the chloracne may have preceded the incident, being present as a result of his routine work. This raises the question of how many of the systemic problems listed were also completely or partially unrelated to TCDD. From the data available, the question cannot be answered.

TABLE 9

Organ Systems Reported Affected After Exposure to TCP and TCDD Following an Industrial Accident

Source	Number Examined	Skin	Liver	Urinary Tract	Lower Respiratory	Mucous Membranes	Central Nervous System	Peripheral Nervous System	Autonomic Nervous System	Heart	Pancreas	Special Senses	Comments
Dugois et al. (25)	21	21[a]											
Jensen and Walker (36)	83	83	13	1		+[b]							Includes family members f exposed worker
Goldman (29,30)	42	42	6	1	7	4	1	6	6	1	1	3	Includes 14-yr old son of employee
Anonymous (2) (Seveso, Italy)	176	1	29		28					9			Includes only workers at ICMESA Plant
Suskind (75)	228	+	+			+	+	+					
Number of cases in which organ system affected[c]	550	147	48	2	35	4	1	6	6	10	1	3	

[a] Number entries in table reflect the number of cases in which a disorder of the organ system was reported.

[b] + = Organ system involvement reported. Number of cases not given.

[c] Numbers do not include cases represented by "+".

TABLE 10
Signs, Symptons and Disorders Reported After Exposure to TCP and TCDD Following an Industrial Accident

Source	Headaches	Neuritis	Myalgia or Neuralgia	Paresis	Hemorrhage	Porphyria	Pigmentation or Hirsutism	Acne	Asthenia	Abdominal Pain or Pressure
Dugois, P. et al. (25)								21[a]		+[b]
Goldmann (29,30)	5	1	6	7	+			42	3	
Reggiani (64)								1	11	
Suskind (75)	+	+	+	+		+	+	+	+	+
Number of cases reported[c]	5	1	6	7	0	0	0	64	14	0

[a] Number entries in table reflect number of cases in which sign, symptom or disorder was reported.

[b] + = Sign, symptom or disorder reported.

[c] Numbers do not include cases represented by "+".

TABLE 11

Special Clinical Studies After Exposure to TCP and TCDD Following an Industrial Accident

Source	Liver Funct	Renal Funct	Carbohydrates	Lipids	Blood Pressure
May, G. (53)	13[a]	1	3		
Goldmann (29,30)	+	+		+	
Reggiani (64)	+[b]				17
Suskind (75)	+			+	
Total number of cases with abnormal study[c]	13	1	3	0	17

[a] Number of entries in table reflect the number of cases in which the special study was reported as abnormal.

[b] Special study reported as abnormal but number of cases not given.

[c] Numbers do not include cases represented by "+".

Still, even with the limitations the data do allow for an evaluation of trends. Chloracne is by far the most common finding. Also appearing frequently are disorders involving the liver, nervous systems, and mental state, the latter primarily in the form of asthenia. Early symptoms such as respiratory tract and mucous membrane irritation as well as headaches and nausea probably result from the primary substance and not TCDD (75). Lipids are frequently evaluated, but due to the normal large day-to-day variation within an individual, the findings are difficult to evaluate. Chloracne, asthenia, and liver disease in the form of porphyria cutanea tarda will be discussed in greater detail.

a. Chloracne. Chloracne is the hallmark of exposure to the highly chlorinated dibenzodioxins and dibenzofurans. Kimmig and Schulz (44, 45) in 1957 and Schulz in 1968 (73) showed that it was TCDD and not TCP that produced chloracne. The history of chloracne since its first description in the late 1800's has been well documented in numerous review articles (16, 21, 43, 44, 74, 77). The problem peaked about the time of World War II as the result of a large production of chlorinated napthalenes. It also has been a major problem with the polychlorinated biphenyls and the associated dibenzofurans, particularly in Japan. The incidence of chloracne has been decreasing as production techniques and housekeeping methods have improved resulting in a reduced level of TCDD.

Chloracne is a disorder of the pilosebaceous mechanism with the overproduction of keratin in the sebaceous ducts. This results in the development of the comedone or blackhead seen in all types of acne. In mild cases this may represent the full extent of the disorder. However, the natural progression is the formation of cysts and in severe cases to the development of inflammatory lesions and scar formation. Inflammation, however, tends to be less prominent than that found in acne vulgaris (common or juvenile acne). Frequently associated with the chloracne are hyperpigmentation and hirsutism manifested by excessive facial and body hair.

In the mildest cases acne may only appear in the area of the outer canthus of the eye and pre- and post-auricular regions. In somewhat more severe cases, the rest of the face and neck may be involved with a sparing of the nose. In even more pronounced cases, the trunk and extremities, except for the hands and feet, may be affected. A preferential site of involvement not usually seen in other forms of acne is the genital region. In the worst cases the skin of the entire body gives the appearance of a homogeneous covering of comedones and small cysts. Severity of the acne does not necessarily reflect the degree of exposure to TCDD (14).

Acne may appear as early as two to three weeks after the first exposure; however, there may be a delay of several months. The delay could represent a time for the development of a skin burden of TCDD. (Personal communication: Holder B. B., Dow Chemical Company, Midland, Michigan.) This burden would represent a threshold below which acne does not appear.

Oliver in 1975 (57) reported two laboratory workers who developed very greasy skin, one of whom developed acne. This picture is contrary to the usual finding in chloracne where the skin is typically very dry. (Personal communication: Crow, K. D., Princess Margaret Hospital, Swindon, England.) Why these workers developed the greasy skin cannot be explained and must at this time be considered an anomaly.

Experience from the industrial episodes (and from the Seveso, Italy episode) confirm that mild chloracne may clear quickly (e.g., in months). Severe chloracne is known, however, for its recidivism. Cases with active lesions have persisted for up to fifteen years after exposure ceased (53).

Many of the studies of systemic effects used populations presenting with chloracne as a point of entry. This probably is not a major weakness, however, because chloracne is one of the earliest indicators of disease (13). Nevertheless, it could have resulted in an artifically lowered incidence of systemic effects present without acne.

b. Porphyria Cutanea Tardia. Porphyria cutanea tarda (PCT) is a disorder of heme pigment metabolism characterized by skin sensitivity, accumulation of excess pigment in the liver, and the build-up of the various porphyrin pigments. Skin findings include skin fragility, bullous lesions, pigmentation, and photosensitivity. It may be either hereditary or acquired. The latter is usually associated with hepatic disorders.

Bleiberg et al (14), in 1964, discovered eleven cases of PCT in workers involved in the production of 2,4,5-trichlorophenol. In 1973, Pazderova et al (61) reported on twenty-three additional cases. Liver biopsies were obtained in five subjects and liver tissue from necropsies in two. All showed fluorescence under ultraviolet light indicating high levels of porphyrins. In 1971, Poland et al (62) studied the workers described by Bleiberg and noted only one asymptomatic urine uroporphyrin. Changes in the plant had greatly decreased the exposure to TCP and TCDD.

The hyperpigmentation and skin lesions found in PCT are independent of those found in chloracne. Pre-existing liver disease appears to predispose a subject to PCT when challenged by another agent such as TCDD.

Based on the majority of studies, systemic disease does not result unless chloracne is present either before or during the course of the disease. However, Bleiberg (14) found porphyria was present in two cases without acne, and Oliver (57) noted that one laboratory worker synthesizing TCDD developed rather severe systemic symptoms but never any acne. It is accepted that chloracne can result from external exposure to TCDD. The role of systemic absorption of TCDD in the development of chloracne has been a matter of debate. Similarly, it is even less clear what role percutaneous absorption of TCDD plays in the development of systemic disease. Regardless, the basic observation is true enough so that an etiology other than TCDD should be diligently searched for in any case where symptoms developed without acne.

c. Asthenia. Many asthenic and other vegetative symptoms have been described in 2,4,5-T, TCP and TCDD intoxication. For purposes of this report, asthenia includes the following: headache, apathy, fatigue, anorexia, weight loss, sleep disturbances, decreased learning ability, decreased memory, dyspepsia, sweating, muscle pain, joint pain and sexual dysfunction. True pathology is closely interwoven with the depression which undoubtedly exists as a result of other disorders, particularly the disfigurement of chloracne, therefore causing difficulty in interpretation of these symptoms. This problem is well demonstrated in a report on polybrominated biphenyl (PBB) exposure in the April 7, 1978, issue of the Center for Disease Control *Morbidity and mortality Weekly Report* (17). Several hundred pounds of PBB were accidently introduced into animal feed. Three cohorts were studied, the first involving all persons who had been identified by the Michigan Department of Public Health as living on PBB contaminated farms at the time of quarantine, the second including persons who had received food products directly from such farms, and the third included workers (and their families) who had been exposed occupationally to PBB in a chemical manufacturing plant. Two additional groups with low level PBB exposure were also evaluated. Highest PBB levels were found in those groups in whom one would expect the exposure to be the greatest. However, symptoms occurred most frequently in volunteers and in persons from nonquarantined farms with low level PBB contamination. Symptoms were least prevalent in quarantined farm families and in chemical workers, just the opposite of what one would expect. Symptoms and conditions included fatigue, rashes, joint pains, hepatitis, diabetes, benign tumors, and cancer. The point to be made is that signs and symptoms of asthenia are common and need not be related to chemical exposure.

There is little question that asthenic symptoms can develop following TCDD exposure. In an early plant accident in which exposure is felt to have been massive, workers developed fatigue and severe muscle pain (75). Impotency was present. As it was one of the first such episodes, the symptoms of TCDD or TCP exposure had not been delineated, and therefore the effect of suggestion would have been minimal. It needs to be emphasized, however, that the exposure was massive and the symptoms did clear. One must be very careful in transposing the results of this accident to another where exposure was much less. One is on particularly tenuous ground if he attempts to attribute the symptoms to the exposure levels found in herbicide spraying.

2. Special Case Studies

a. Exposure resulting from spraying operations. The study by Londono in 1966 (51) is of special interest in that he reported on five subjects who were involved in herbicide spraying as opposed to industrial exposure. The herbicides included the butyl ester of 2,4-D and the methyl ester of 2,4,5-T. All five developed chloracne. One of the workers manifested the acne seventeen days after the onset of spraying, three after two months, and one after eighteen months. No clinical systemic disease was reported, although liver function tests were mildly abnormal.

b. Controlled study on 2,4,5-T plant workers. In contrast with the episodes just described, which were in effect case studies, Kramer in

1970 and revised in 1974 (49) in an unpublished report described a control study on the health of employees exposed to 2,4,5-T at Dow Chemical Company. The control population of 4,600 non-exposed Dow employees did not vary significantly from the general population. Fifty clinical parameters were investigated including both history and laboratory studies. Parameters included were those which would be indicative of disorders of the central nervous system, mucous membrane irritation, pulmonary disease, cardiovascular disease, gastrointestinal and hepatic disorders, renal disease, asthenia, and psychiatric disorders. No significant differences were found between the study and control groups.

3. General Population Exposures

The discussion up to this point has generally related to the occupational hazards of TCP, 2,4,5-T and TCDD. In recent years there has been considerable interest expressed by a number of groups concerning the public health aspects of the phenoxy herbicides and TCDD. The remainder of this section will concentrate on several incidents in which the general population was involved. Chapter V has provided more extensive details on each of these episodes.

a. South Vietnam Episode. In the latter part of 1969 newspapers in South Vietnam reported that there were an unusually large number of malformed babies being born among the Montagnards. This was followed by a publication by Tung et al (85) of the Democratic Republic of Vietnam in 1971 in which they reported 179 people who had lived in sprayed areas from two months to five years or had been in direct contact with the spray. He did not specify the type or composition of the spray material. Disorders were divided into three major groups: asthenia, ocular syndrome and genetic effects. The general asthenia was accompanied by insomnia, headache, sexual impotence and menstrual problems in females. A specific form of the asthenia, visual asthenia, was characterized by early onset of eye fatigue (5-15 minutes) when reading. The ocular syndrome consisted of the visual asthenia (mentioned above) as well as a decrease in visual acuity and corneal scarring. The genetic syndrome consisted of chromosomal alterations in seriously affected adults, congenital malformations (particularly Trisomy 21) in the new born, and unclassifiable multiple congenital malformations with multiple chromosomic alterations.

In 1973, Tung et al (86) reported an increase in the number of persons with primary liver cancer in proportion to all cancer patients admitted to Hanoi hospitals during the period 1972-1968 (790 liver cancer cases out of 7911 cancer cases, 10 percent) as compared to the period 1955-1961 (159 liver cancer cases out of 5492 total cancer cases, 2.9 percent), which was prior to the start of herbicide spraying. The authors attributed this increase to exposure as a result of the spraying of herbicides containing TCDD in South Vietnam during the 1960's; however, a recent IARC monograph (34) noted that limitations in the reporting of the study make impossible an adequate assessment between the incidence of liver cancer and herbicide spraying in South Vietnam.

A National Academy of Science (NAS) committee (18) was established in 1972 to investigate the effects of herbicides in Vietnam. In their report, published in 1974, they described an earlier study by Cutting et al (22) in 1970 reviewing congenital malformations, hydatidiform moles, and stillbirths in 22 hospitals in the Republic of Vietnam for the periods between 1960-1965 and 1966-1969. The first time period involved light spraying of Herbicide Orange, the second, heavy. Neither the NAS nor Cutting et al were able to demonstrate any influence of the herbicides on the development of these disorders.

It must be pointed out that all studies in Vietnam were limited by poor and incomplete reporting, and most important by the politics of the area. Large segments of the population in question were not available to the investigating groups.

b. Eastern Missouri Horse Arena Episode. Following the spraying in 1971 of three horse arenas in Lincoln County, Missouri, with salvage oil contaminated with TCDD, a number of people who worked or played in the arenas developed medical disorders (10, 19, 43, 50). The most serious was a six-year-old girl who developed hemorrhagic cystitis and focal pyelonephritis. The urinary tract symptoms were preceded by headache, epistaxis, diarrhea and a general malaise. Her urinary tract symptoms cleared after a few days. Three months later examination was normal except for punctate hemorrhages of the bladder seen on cystoscopy. The father of the child had developed headaches and nausea while working in the arena. Her mother reported severe headache, nausea, diarrhea and abdominal pains, and arthralgia. A ten-year-old sister developed easy fatigability, epistaxis, headaches, abdominal pain, and diarrhea. All developed at least mild acne lesions (64). Follow-up studies on the mother and two children performed five years later were normal (10). Chloracne developed in two three-year-old boys who played in another arena (19, 43). One case lasted more than a year. Commoner and Scott (19) in 1976 mentioned one additional case of chloracne in a veterinarian who obtained samples in a third arena.

The symptoms in all seven of the humans were relatively mild with the most severe being the hemorrhagic cystitis and focal pyelonephritis seen in the six-year-old girl. The symptoms had cleared on re-examination several years later. Exposure, at least to the four children must have been significant in that they regularly played in the soil of the arenas. Contrast this with the disastrous effects the dioxin had on the horses and other animals that were in the arena often for only a short period (43). This gives strong support to the contention that man is relatively resistant to TCDD or absorbs it to a much lesser extent.

c. The Seveso, Italy Episode. The details of the Seveso, Italy, incident where an industrial accident resulted in the exposure of the general population to a cloud of TCP and other toxicants are described in the previous chapter. This incident is most significant in that it represents the first episode where a cross section of a community received a definite exposure to TCDD, although the degree of exposure can only be estimated. As in any such situation confusion reigns, and a great deal of information is passed consisting of a mixture of truths, half-truths, and untruths. This confusion was amplified by the fact that the caustic nature of the cloud produced serious irritative effects including skin burns in many of the people who came in direct contact with the cloud. Very early after the incident an organized program was established to follow the general populace to determine what if any effects, both long and short-term, resulted (26). The findings for the first two years have been reported and are summarized as follows (2, 64, 65, 83, 84):

(1) Chloracne--A massive screening program was initiated in 32,000 school children below the age of ten. Seventy-nine cases of chloracne were confirmed, only eight of which were severe. Many of the victims were not present in the area until weeks or months after the accident, indicating that the TCDD remained in the environment outside the zone of evacuation at a level high enough to produce chloracne. Except for the eight severe cases, the acne cleared in a few months. Two years after exposure some of the severe cases still showed active lesions and had severe scarring (65). In October 1977, six new cases were found in children who returned to their homes after decontamination, bringing the total to 85. No systemic abnormalities have been found in the children with acne.

It is of interest that nearly all cases of acne were found in children. There are several possible explanations for this. A massive systematic search for chloracne was undertaken for children under the age of ten. No such search was undertaken for adults. It may be that children are more sensitive than adults. This is a phenomenon frequently seen with chemicals and drugs. It may also simply be that the children's daily routine results in greater exposure.

(2) Spontaneous Abortion and Fetal Malformation--Information concerning the birthrate, abortions and fetal malformations for the two years following the accident revealed no significant changes (2, 64, 65, 83, 84). There were several problems regarding the evaluation of the results. First and most important was the lack of reliable background data for the area. Worldwide figures and figures from the Lombardy region of which Seveso is a part were used. Data were also biased by the fact that therapeutic abortions were offered to women who were pregnant at the time of or immediately after the accident. Nevertheless, it was the opinion of the evaluators that significant increases in spontaneous abortions and fetal malformations could not be demonstrated. Chromosomal studies were performed on the fetuses of thirty pregnancies interrupted between August 13 and December 10, 1976. No abnormalities in number of pattern beyond the expected rate were seen (2, 64, 65, 83, 84).

(3) Immunology--No differences in immunoglobulins and B lymphocytes were found between a study and a control group of children, even though twenty of the children in the study group had chloracne (65). Hospital admissions and disease classification data evaluation revealed no significant changes from the previous year. There was an increase in

infectious diseases compared to the previous year, but this increase was also seen in the nonexposed districts (2, 64, 65).

(4) Summary--Except for the initial irritative effects of the caustic substances and the presence of eighty-five cases of chloracne, no adverse effects to the chemicals in the toxic cloud have been confirmed. It must be remembered, however, that in many instances the findings were not conclusive and that long-term effects such as cancer and hidden congenital malformations have not yet had time to manifest themselves. It will be several years before all the data are published on the Seveso incident.

d. Globe, Arizona Episode. In 1969, a number of residents in the Globe, Arizona area alleged that numerous physical ailments resulted from the spraying of the surrounding area with 2,4-D, 2,4,5-T and Silvex. Symptoms and disorders mentioned included headaches, fatigue, chest and arm pain, worsening of pre-existing nasal allergies and asthma, loss of the sense of smell and taste, severe diarrhea, spasms of the arms and legs, anemia, irregular and painful menses, spontaneous abortions, fetal malformations and cancer (82). An investigation in 1970 by Tschirley et al (82) was unable to connect the disorders with definite exposure; however, he recommended further studies. As a result of this recommendation Roan and Morgan (66) in 1972 reported on the results of an epidemiological study of the hospital records in the area and a pesticide analysis of several body tissues and fluids including adipose tissue. The technique of analysis allowed minimum detection of TCDD at 2 ng/gm tissue and of 2,4,5-T, 2,4-D or Silvex at 0.01 ng/gm tissue. No 2,4,5-T, 2,4-D, Silvex or TCDD was found. They concluded that the probability of chronic human exposure in the area in question was very minimal.

e. The Swedish Lapland Episode. As noted in Chapter V, this episode involved a series of public debates on the risks to humans (and animals) of the phenoxy herbicides, especially 2,4,5-T. In the March 1978 WBBM television report on "Agent Orange: Vietnam's Deadly Fog," reference was made to a report from Sweden on birth defects (e.g., spina bifida) in children born to 65 women allegedly exposed to 2,4,5-T herbicide. The only reference to such an incident was that reported by Halling (32) in 1977. Halling reported on the presence of malformations in children born to mothers exposed to hexachlorophene soap during early pregnancy. A group of 65 children born to this group showed six slight and five severe malformations. This contrasted with one slight malformation in 68 children born to a group of nonexposed mothers. It needs to be emphasized that the chemical in question was hexachlorophene and not phenoxy herbicide.

f. Te Awamutu, New Zealand Episode. In 1972 Sare and Forbes (68) reported on two babies born within a month of each other in the same hospital, each presenting with meningomyelocoeles. They lived in farm country where spraying with 2,4,5-T had been routinely carried out for several years. The possibility that the malformations may have been related to the herbicide was suggested. Because of this report and other allegations that neural tube deformities were the result of 2,4,5-T exposure, a thorough, although retrospective, investigation of the problem was undertaken by the

Department of Health in 1977. The investigating committee (1) concluded that there was no evidence to implicate 2,4,5-T as an etiologic factor.

D. Cancer

There are a number of individual case reports and geographically limited studies of herbicide workers, both in manufacturing as well as application, that suggest an associative relationship between exposure to either 2,4,5-T, TCP or TCDD and subsequent development of a variety of neoplasms.

Of 75 workers exposed in a TCP factory accident in 1953, most were affected by chloracne, 42 were listed as severe. All of the 75 workers could be traced 25 years later and while the mortality rate was no higher than expected, there had been 6 deaths due to cancer versus the 4 that could have been expected from national averages. Three of the deaths were due to stomach cancer in the 60-69 year age group, which was significantly more than expected (35).

One worker involved in an accident in a 2,4,5-T producing factory in the Netherlands in 1963 died of carcinoma of the pancreas in 1964 (35). Because of the extremely short time span between the exposure and the death, it is not likely that the two are related.

In 1973 Tung (86) reported an increased incidence of hepatic cancer in Vietnamese allegedly exposed to the spray of Herbicide Orange. A lack of details in the reporting make evaluation of the claim difficult.

Jirasek et al (37, 38) and Pazderova et al (61) reported the presence of two bronchiogenic carcinomas at ages 47 and 59 during the first five years of the follow-up of 75 workers occupationally exposed to 2,4,5-T and pentachlorophenol. They noted that only 0.12 lung cancer deaths were expected from national mortality statistics. Again, the latent period was short and no smoking statistics were given.

In 1972, newspapers in Sweden reported an excess mortality due to lung cancer in railroad workers exposed to herbicides. As a result, Axelson and Sundell (5) initiated a controlled study and in 1974 reported that although there appeared to be an increased incidence with Amitrol there was no significant increase with 2,4-D or 2,4,5-T. However, a re-evaluation of the data indicated a possible and previously masked tumor inducing effect from the phenoxy acids (35).

A similar study on workers involved with spraying 2,4-D and 2,4,5-T on brushwood in Finland showed no increase in overall mortality. There were, however, four cases of cancer in the age group younger than 45 years as opposed to the expected two(35).

Hardell (33) reported that of 87 mesenchymal tumors seen from 1970-1976 in the Department of Oncology, Regional Hospital, Umea, Sweden, 19 had been in men whose employment (farmers and forestry workers) may have resulted in exposure to the phenoxy herbicides. The expected mesenchymal cancers for this group was eleven. Seven cases with known sporadic herbicide exposure 10-20 years before diagnosis were presented. Hardell noted the difficulty in establishing a causal relationship but suggested that the deviation from national averages for these relatively uncommon tumors could perhaps be linked to extensive use of the phenoxy herbicides in the Umea region.

Five leukemia deaths have been reported in the area of Meda, Italy, since the Seveso episode in July 1976. No more than 1.4 were expected. One of the cases was found to predate the accident (35). Additionally, the interval from exposure to diagnosis appears to be too short to ascribe causation.

The case of pancreatic carcinoma in the 55-year-old woman described by Reggiani (65) and mentioned previously in the section on the pharmacodynamics of TCDD was also felt not to be related to the exposure to TCDD. To quote Reggiani "A causal relationship with the malignancy can be excluded owing to the lapse of time required by tumor growth to reach the size, weight and diffusion of this case. The exposure to TCDD has occurred at a time when the growth of the tumor had already reached the stage of occult spreading throughout lymphatic and blood vessels to the adjacent tissues and organs." (65)

As noted above, these studies should be viewed only as preliminary evidence of a statistical relationship between exposure to the phenoxy herbicides, TCDD and TCP and subsequent cancer development. Except in cases such as the angiosarcoma caused by vinyl chloride where the type of cancer is rare and the association with exposure irrefutable, it is virtually impossible to differentiate a cancer caused by a specific chemical agent from a similar cancer caused by some other etiology. This is certainly true with the retrospective studies currently available and may be true even with meticulously controlled prospective studies. There are, however, a number of cohort studies either ongoing or planned which may help clarify the present uncertainty concerning the role of the phenoxy herbicides in cancer causation in humans (35).

IV. CONCLUSIONS

A. Pharmacodynamics

1. 2,4-D and 2,4,5-T are readily absorbed via the cutaneous, and gastrointestinal routes, distributing throughout the body. The respiratory tract may also be a point of entry although of lesser importance.

2. Liquid phenoxy-herbicide contact to the skin can produce systemic reactions.

3. 2,4-D and 2,4,5-T have relatively short half-lives in the human body and persistent body burden is unlikely to develop, at least in short-term or intermittent exposures.

4. Other than the knowledge that TCDD may enter the body percutaneously, the pharmacokinetics of TCDD in man are essentially unknown. Based on the way other pesticides are handled, it is reasonable to assume that the use of 2,4,5-T has resulted in considerable skin-liquid contact. In spite of this, reports of 2,4,5-T toxicity and therefore TCDD toxicity are minimal considering the degree of use. This may indicate that man is more resistant to the effects of 2,4,5-T and TCDD than other animals, but it could also indicate that percutaneous absorption is less. The apparent relative lack of toxicity or percutaneous absorption is further supported by the Missouri incident where there was a marked difference between the degree of toxicity in man and animals.

B. Effects of the Herbicides

1. The use of 2,4-D and 2,4,5-T worldwide since the middle 1940s with minimal reports of adverse effects indicate that they are generally safe chemicals if properly used. Large total doses of 2,4-D have been given to humans in controlled circumstances without adverse effects.

2. The nervous system is particularly sensitive to 2,4-D. If peripheral neuropathy developed following exposure to 2,4-D, it normally disappears in a matter of months. However, in some reported incidents, it did persist for one to three years.

3. Symptoms present within the first few days after exposure are probably due to the herbicide and not TCDD.

4. Adverse effects of 2,4-D and 2,4,5-T should manifest themselves shortly after exposure. Symptoms arising for the first time, months to years after the last exposure are probably due to an etiology other than 2,4-D and 2,4,5-T.

5. The hematopoetic system may be an important target organ for 2,4-D in some people.

C. Effects of TCDD

1. If there is not a history of chloracne, it is highly unlikely that systemic changes will be due to TCDD. However, the acne may be minimal and, therefore, the historical search must be meticulous.

2. The presence of active chloracne months to years after exposure does not necessarily mean continuing exposure.

3. Skin lesions of porphyria cutanea tarda are independent of those associated with chloracne.

4. The development of porphyria cutanea tarda following exposure to TCDD suggests an adverse liver response to the TCDD.

5. Although asthenia is difficult to interpret, it probably represents a symptom of TCDD intoxication.

6. A rise in serum lipids may occur after exposure to TCDD. However, because of large individual variations, the finding is difficult to interpret.

7. Claims of carcinogensis, teratogenesis, and mutagenesis in man have not been confirmed at this time for the phenoxy herbicides or TCDD. However, the topic remains open.

8. The preliminary information from the Seveso episode and the study by Kramer on the health of 2,4,5-T workers indicate that incidental nonoccupational exposure to small amounts of TCDD is unlikely to produce symptoms.

9. The long-term effects of large acute doses of TCDD or small intermittent or chronic exposures are not known.

V. SUMMARY

The pharmacodynamics and adverse effects of the phenoxy herbicides, trichlorophenol and TCDD were reviewed, primarily through reports of occupational exposure and accidents as well as reported exposures to the general public. A number of organ systems may be involved if the dose is significantly high with emphasis on the skin, liver, CNS and peripheral nervous system. Adverse effects of 2,4-D and 2,4,5-T should manifest themselves shortly after exposure. Symptoms arising for the first time months to years after the last exposure are probably due to an etiology other than 2,4-D and 2,4,5-T. The hallmark of TCDD is chloracne and its absence makes it unlikely that systemic disorders present are related to TCDD. Asthenic and vegetative symptoms are often present in overexposure but are difficult to interpret. They would normally be expected to clear with time. There is no conclusive evidence at this time that the phenoxy herbicides or TCDD are mutagenic, teratogenic or carcinogenic in man.

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